

**The germ cell history of *Rana*
cantabrigensis Baird**
II. Sex differentiation and development

By

Tso-Hsin Cheng

With 32 figures in the text

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THE GERM CELL HISTORY OF RANA CANTABRIGENSIS BAIRD.
II. SEX DIFFERENTIATION AND DEVELOPMENT¹.

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With 32 figures in the text.

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¹ Contribution from the Zoölogical Laboratory of the University of Michigan.

In *Rana cantabrigensis*, following the formation of typical indifferent gonads, as described in a previous publication (CHENG, 1932), the germ cells or the primitive gonia soon enter upon a process of morphological sex differentiation. This is accompanied by, or rather associated with characteristic changes in the structures and organization of the gonad. Extra-gonadal sex characters do not develop until near metamorphosis, or thereafter. In describing the course and processes of sex differentiation and development in this frog species, it is found convenient to divide the history into three consecutive periods, namely, larval, post-larval and adult. Sexual maturity is attained in the adult period, during which time there is a complete development of all structures, germinal as well as somatic, which are regarded as characteristic of the sex.

1. Larval Period.

The development of germ cells and gonads during this period has been followed in other species of frogs, e. g., *Rana temporaria* (BOUIN 1901; WITSCHI 1914), *Rana esculenta* (KUSCHAKEWITSCH 1910), and *Rana catesbeiana* (SWINGLE 1921—26). More recently, WITSCHI (1929 a) has published a brief account of the development and sex differentiation of gonads in *Rana sylvatica*, which is confirmatory in nature to his previous work on *Rana temporaria*, but is, in many respects, insufficient in essential details. While the developmental conditions of germ cells in *Rana cantabrigensis*, the so-called northern wood-frog, are similar to those described for *Rana sylvatica* or the eastern wood-frog, and for differentiated races of other frog species, special consideration will be given, in the following account, to certain features, which are distinctive of our species, and which have apparently been overlooked or only casually mentioned by previous investigators on this problem.

A. Male Development.

a) *Gonad proper (Testis)*. Gross structure: In tadpoles of about 50 days in age, the testes can be macroscopically identified as small,

elongated and more or less cylindrical structures, located at the cranial ends of the mesonephroi, and attached to their ventro-medial margins (fig. 1). Owing to the segmental arrangement of rete cords, younger testes have a metameric appearance which soon fades out as the rete cords enlarge and anastomose with one another. Measurements of scores of testes in serial sections, part of which data is presented in table 1, show that during the larval period, the left testis is generally longer than



Fig. 1. Early testes in a tadpole 51 days old and 33 mm. total length.



Fig. 2. Testes of a metamorphosing male 75 days old.

the right one, and extends further either anteriorly or posteriorly or in both directions beyond the limits of the right testis. Toward and during metamorphosis, the right testis becomes, in many cases, shifted cranially of the corresponding left one. Both testes during this time have practically assumed, as shown in figure 2, the form characteristic of the adult testes, being more or less rounded in transverse sections, and appearing somewhat ovoid in shape longitudinally, with a well-developed corpus adiposum in front and a long rudimentary postgonad behind (fig. 3). In proportion to their size, the testes of metamorphosing and newly

Table 1. *Length Measurements of Male Gonads.*

Specimens			Length of gonads in mm.					
Age in days	Length in mm.		Progonads (Fat-bodies)		Gonads proper (Testes)		Postgonads	
			Lt	Rt	Lt	Rt	Lt	Rt
1.	21	17	0.19	0.07	0.32	0.30	0.07	0.06
2.	26	19	0.26	0.10	0.38	0.38	0.11	0.11
3.	30	25	0.61	0.26	0.30	0.24	0.44	0.24
4.	30	28	0.65	0.49	0.40	0.27	0.33	0.36
5.	38	30	0.58	0.27	0.34	0.42	0.27	0.27
6.	44	34	0.70	0.56	0.28	0.28	0.36	0.28
7.	51	35	1.18	0.95	0.53	0.50	0.36	0.37
8.	51	42	1.25	1.28	0.48	0.55	0.51	0.14
9.	59	48	1.43	1.30	0.57	0.33	0.35	0.09
10.	66	39	0.79	0.78	0.51	0.34	0.40	0.23
11.	66	Metamorphosing	—	—	0.53	0.37	0.23	0.09
12.	72	"	—	—	0.41	0.53	0.88	0.25
13.	78	"	—	—	0.51	0.52	0.40	0.62

metamorphosed frogs appear to be shorter than larval testes before the time of metamorphosis. From actual measurements (table 1), this shortening is shown to be relative rather than actual. Throughout the entire larval period, the testes show no external pigmentation, as has been found in *Rana temporaria* by WITSCHI (1914), and *Rana pipiens* by CHRISTENSEN (1930).

Microscopical structure: The histogenesis of the testis during larval development and the morphogenetic features of the cells concerned may be presented in stages as outlined and described below.



Fig. 3. Tadpole 62 days old. Longi-section of the left testis with a portion of the fat-body in front and the long postgonad behind.

1. *Larva 21 days old and 17 mm. total length.* Both gonads are essentially of the indifferent type, with a cortical germinal layer which encloses the rete-cord and intercordal tissues and is itself enclosed by a delicate surface peritoneum. The growth of the gonad at this time is mostly due to the rapid increase in volume of rete cords through mitoses of their component cells, and through additions of mesenchymal elements derived from a nephrogenous origin. The primary genital cavity is being incorporated into the growing rete cords.

The gonocytes are of varying sizes, and contain varied amounts of vitelline granules. Some cells are still packed full of yolk, and measure 25—30 micra in diameter. Those devoid of yolk are smaller in size, ranging from 14 to 20 micra. The decrease in size is evidently due to the absorption of bulky yolk material, and probably also to the rapidly repeated mitoses of the cells in question. Mitotic figures are encountered in the germ cells, even in those which still contain some large yolk spherules. The protoplasm of

the germ cells stains lightly and shows minute granules, which are scattered in the juxta-nuclear region and are, when present in large quantities, often more or less massed together along one side of the nucleus.

The germ cells are mostly confined to the cortical portion of the gonad. In the right gonad, there are a few scattered germ cells migrating from the gonadic cortex toward the rete cord to become attached to its peripheral portion. These cells are accompanied by their follicles,

delicate and yet distinct. Figure 4 shows one of such migrating gonocytes, and the space in the cortex, which it previously occupied. There has been much argument as to whether this migration is an active or a passive process (WITSCHI 1914; SWINGLE 1926). It is obvious that whatever the actual mechanism may be, this process is distinctly a response on the part of the germ cells to the predominating sex tendency. The mere fact that this migration always takes place with reference to the rete cord, shows that the processes here concerned are under the activating influence of the rete-cord substance. The rapid growth of rete cords and the association of germ cells with them are, as will be more clearly shown in later development, the characteristic features of the male sex.

2. *Larvae 25—30 days old and 20—25 mm. total length.* With rare exceptions, the germ cells are at this stage entirely free from yolk, though still containing varying amounts of cytoplasmic granules. Their nuclei are either more or less rounded with or without slight notches here and there, or markedly lobated, appearing as solid clusters of more or less separate elements. In some cases, the nucleoli may be one or few and are large, measuring 3—5

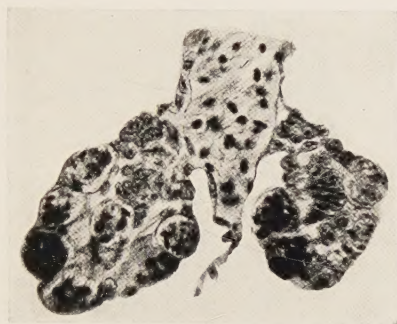


Fig. 4. Transection of gonads of a tadpole 21 days old and 17 mm. total length. Note a yolk-bearing germ cell in the rete cord of the right gonad, which is differentiating in the male direction.

micra in diameter. In most of the polymorphic nuclei, however, the nucleoli are smaller in size and more numerous. Their average size is about 1 micron, and their number ranges from 3 to 10 or more. In either condition, the nucleoli stain intensely black with iron-hematoxylin, and are spherical in form. Their number and size, in every case, seem to be indicative of the metabolic condition or physiological activity of the cells concerned. It is important to note that nuclear polymorphism so characteristic of the early germ cells of our frogs, and probably of all amphibians, is not a sign of fragmentation or degeneration of the nucleus. In none of the lobulated nuclei, have we detected any indication of chromatolysis or of any other form of abnormality. The chromatic substance is in the form of minute, faintly-stained granules collected in irregular strands, which are condensed along the periphery of the nucleus and around the nucleoli, and are elsewhere loosely distributed throughout the more oxyphilic karyolymph. The cytoplasm is small in amount, in proportion to the size of the nucleus. In sharp contrast to the germ cells, the stroma tissue of the germ gland is characterized by small, compact and relatively deeply stained nuclei in a cytoplasmic

syncytium. These nuclei may be rounded, elongated, or rarely conical, but never polymorphic. They contain numerous indistinct and irregular clumps and streaks of chromatin, and among them, one or two or rarely more nucleoli, all of which are smaller and less conspicuous than those in the germ-cell nucleus. The remarkable difference between germ cells and the mesodermal cells of the stroma appear to exclude all possibility of any transformation among them in either direction.

Germ cells are frequently seen in mitoses, during which all of the nucleoli disappear and the chromatin substance resolves itself into

heavy spiremes which during late prophase and metaphase become condensed into definitive thick chromosomes arranged on a large achromatic spindle. They can in no way be confused with the much smaller and less conspicuous mitotic figures occurring in the somatic cells of the stromal syncytium.

Many germ cells are found migrating inward to the rete tissue, penetrating deeply into the latter. They occur either singly or in loose association with one another, but are always isolated by distinct follicles. In the most posterior portions of the testes, germ cells are often found included in the cortical region, while elsewhere the inward migration is in full course (fig. 5). The evidence seems convincing that the process of gonadic sex differentiation generally proceeds in a craniocaudal direction.

With the progress of germ-cell migration to the rete tissue, the cortical portion becomes, sooner or later, deprived of all or most of the germ cells, and



Fig. 5. Longisection of a differentiating testis in a larva 30 days old and 25 mm. total length. Arrows indicate the position of rete cords. In the anterior cords there are many germ cells, while the most posterior cords are small and contain no germ cells. The primary genital cavity shows distinctly in the caudal portion of the gonad.

the scanty mesenchymal elements become arranged into a thin layer of connective tissue, which, in position, is comparable to the tunica albuginea of mammalian testes. This cortical connectivum is loose in organization, and is closely adherent to the peripheral layer of peritoneal cells. The nuclei of both layers are fusiform and attenuated, and are arranged with their long axes parallel to the surface of the gonad.

3. *Larvae 30—45 days old and 25—35 mm. total length.* Testes of this period show increasing numbers of gonocytes with relatively scanty stroma tissue interposed among them. In younger testes already described, the condition is precisely the reverse: Germ cells are few and

scattered among the crowded rete elements. This may plausibly indicate that the growth of the gonad during this stage is due more to the rapid multiplication of germinal components than to an increase of somatic tissues.

The segmentally arranged rete cords, now heavily laden with germinal cells, gradually anastomose with one another to form a solid homogeneous mass of tissue, extending the entire length of the testis. The germ cells are more or less uniformly distributed throughout the rete mass, without showing any tendency toward local aggregation, although adjacent cells tend to associate themselves in groups, a phenomenon which forecasts the formation of testicular ampullae. The primary genital cavity is nearly completely obliterated, appearing, here and there in younger specimens, as tiny spaces between the rete mass and the peripheral covering of the gonad. The mesenchymatous tissue of the cavity is no longer identifiable as such, and is apparently merged with the rete tissue, though it may in part contribute to the formation of the cortical connectivum.

With closely repeated mitoses, the germ cells often become greatly reduced in size, measuring between 10 and 16 micra. The nuclei of the smallest gonocytes are similar in size to the larger nuclei of the surrounding rete tissue, but the former can always be distinguished from the latter by their oxyphilous staining reaction. Moreover, the germ-cell nuclei are generally polymorphic, and the nucleoli are always distinct and spherical in shape. The rete nuclei, on the other hand, never show any lobulation, and their contents appear dark and indistinct, with one or more inconspicuous nucleoli. The cytoplasmic granules so abundant in the germ cells of the preceding stages, have diminished greatly in number and are present in quantity only in a few cells which appear to be the least active in multiplication, and which are often seen stranded in the cortical connectivum or in or adjacent to the hilar region. The follicle cells are frequently concealed in the numerous interfollicular stroma elements. In a less crowded condition, they are readily recognizable as forming intact and distinct envelopes around the germ cells.

While during this period, germ cells are massed in the rete-cord region, scattered ones, singly or in small groups, have been found, in sporadic cases isolated in the peripheral or cortical region. These cells are apparently delayed in their inward migration to the rete tissue. They may soon become incorporated into the growing rete mass, or much later. In the latter case, they remain in the cortex and undergo multiplication as the spermatogonial cells of the rete-cord region. It is important to note that the cortical germ cells in the testis do not show any characteristics of the female cells, which, as will be later described, are mostly confined to the cortex of the developing ovary.

In a number of testes, otherwise normal, maturation cells are found grouped in or near the cortical region, or rarely scattered throughout

the medullary tissue. These cells are not male sex cells undergoing prematuration, since male tadpoles of this species do not undergo any precocious or abortive maturation cycles during the larval period, a fact already noted in a previous publication (CHENG 1930). In synizetic and presynizetic features, these cells are different from the spermatocytes of similar stages, and are strikingly similar to the young oöcytes found in the larval ovary of similar age. Hence, they should appropriately be regarded as female germ cells and the testes containing them as intersexual. It is considered probable from our previous work and from data presented elsewhere in this paper that these testis-oöcytes, despite their abnormal position in development, might persist for a considerable time and develop into typical ova as found in some abnormal testes in later stages of larval development or in the postlarval or adult period (CHENG 1930).

The migration of nephrogenous cells into the developing testis is still evident in different places, though much less pronounced than at earlier stages. The migratory cells accumulate in the proximal portion of the gonad, and in the mesorchia, where they occur in segmental masses and constitute the anlagen of the extra-gonadal rete apparatus.

4. *Larvae 50—78 days old and 35—50 mm. total length.* Older specimens of this age group are undergoing metamorphosis or are fully developed tadpoles ready to undergo metamorphosis.

The germ cells, as in preceding stages, remain in multiplication, and show no apparent changes in their structure. Histologically, they become grouped into cysts, a process accompanied by a reorganization of the associated stromal elements to form the investing follicles. The first cysts to be recognized consist of 4 or 5 cells, which are so closely pressed against one another that they appear to form a germinal syncytium. After good fixation and staining, however, individual cells are always seen enclosed by delicate membranes, or by a condensation of the peripheral plasma closely resembling a membrane. Cells of the same cyst are probably derived by division of the same parent cell, and the follicle cells, which in earlier stages are seen surrounding the individual gonocytes, apparently participate in the formation of the cyst follicle. As development proceeds, the germ-cell cyst gradually enlarges and assumes the characteristics of testicular ampullae. In metamorphosing tadpoles, the ampullae are well developed and appear as elongated lobules arranged radially around a central main cord of rete cells, which sends branches into the intervening spaces between the individual ampullae. The ampullae enlarge steadily in size and soon acquire lumina with the contained germ cells arranged along the periphery, thus assuming the characteristic appearance of the seminiferous tubules. The cortical connectivum is not always intact, and part of it has apparently merged with the covering epithelium of the deve-

veloping spermatatic tubules. By rearrangement of the rete cells, a network of minute tubules (rete efferentia) is formed, the lumina of which constitute the secondary genital cavity in the testis. In extragonadal rete

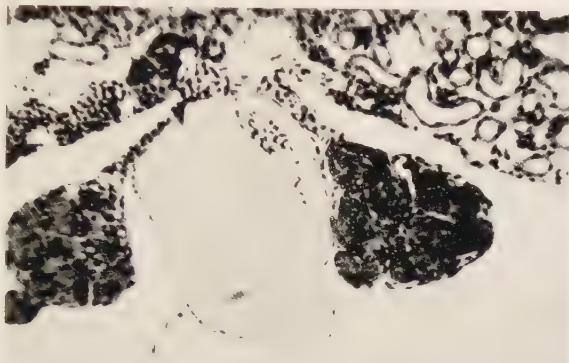


Fig. 6. Tadpole 65 days old. Section shows a protruding lobe of the right testis.

masses, the nuclei frequently appear to be arranged in the form of tubules which usually do not acquire lumina until the time of metamorphosis.

There is only slight, if any, migration of additional rete elements into the gonad, as the latter is extremely dense and compact in structure, and the germinal elements contained therein appear as though they were overcrowded in some places. In a number of cases, germ cells are seen projecting beyond the general outline of the testis, carrying the gonad peritoneum with them as an enveloping fold (fig. 6). These protruding structures may further enlarge, and the testes eventually assume a bilobed condition (fig. 7), a peculiar appearance as compared with the figures of normal testes (figs. 1, 2, 21, 22).

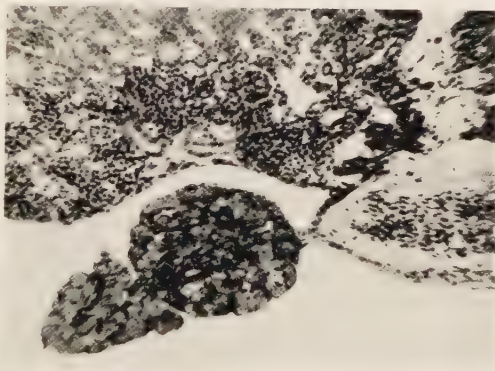


Fig. 7. A bi-lobed testis of a metamorphosing male.

In rare instances throughout the testis development, single spermatogonia or small groups of such cells, enclosed in delicate follicles and normal in every respect, have been found in the process of being extruded into the visceral cavity. Occasionally, expelled germ cells are found in the coelom, but still attached to the gonad periphery (figs. 8 and 9). Careful study makes it evident that this expulsion of germ cells can not be

regarded as an artifact, but that it is an actual condition probably induced by certain growth abnormalities in the development of testes.

At this as well as preceding stages, ectopic germ cells or follicles of germ cells have been encountered in the pro- and post-gonad, within

the mesorchial mesothelia, and in the ventral wall of the postcava adjacent to the dorsal attachment of the mes-orchium. In some specimens, there are found below the level of the postcava at the base of the dorsalmesentery, small follicles composed of several germ cells. Judging from their position, these germinal masses are apparently the remains of the median germ cords, which have failed to migrate to the gonadogenic region. Such an ectopic germ-cell mass is undoubtedly the embryonic foundation of testis heteropia as reported for adult frogs (NUSSBAUM 1906; GERHARTZ 1906; DAUVART 1926, 1927).

Degenerating germ cells have been occasionally encountered in otherwise normal testes. Some of these cells show hypertrophy of the protoplasmic content to an abnormal size and shape, with, however, no apparent degenerative changes in the nucleus. Other degenerative cells show

Fig. 8. Tadpole 70 days old and 41 mm. total length. Transection of a testis, showing an expelled germ cell.

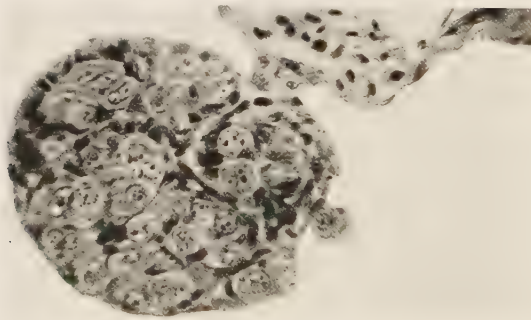
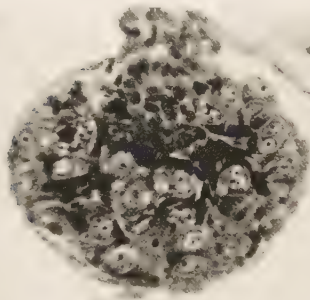


Fig. 9. Tadpole 60 days old. Note an apparently normal germ cell in the process of expulsion from the testes.

shrunk cytoplasm and nuclei which stain darker than usual, and darker still as they become gradually absorbed. In some cases the nuclear materials become aggregated into numerous darkly stained bodies or strands of large size scattered throughout the cell. The whole structure looks like an agglomeration of cellular debris without any organization whatsoever. Eventually the degenerating element becomes

resorbed, leaving an empty space which soon disappears through replacement by the surrounding normal tissue. In the right gonad of a male specimen 72 days old, and in the left testes of two metamorphosing tadpoles there are found varying numbers of enlarged cells in or among the germ-cell cysts, scattered along the gonad periphery or embedded in the rete tissue. These cells measure from about 20 to 34 micra in diameter, and their nuclei resemble those of young or abortive ova in being large and rounded, and showing many nucleoli and a faintly stained chromatin reticulum (fig. 10). Normal spermatogonia in these gonads average about 15 micra in size and contain lightly staining polymorphic nucleoli. It is conceivable that the ovum-like structures just described may be abnormal male cells undergoing oviform hypertrophy in course of degeneration. This seems quite convincing as oviform cells of various sizes can be arranged in a series showing gradual hypertrophy. From an embryological standpoint, however, it is considered probable that the ovum-like cells may represent a further development of the maturation cells found in earlier stages. A similar specimen has been previously reported (CHENG 1930), in which case the oviform cells are, on the average, larger and more numerous. It was then assumed that they were the remains of an ovarian tissue, destined later to degenerate completely. It was also pointed out that an oviform appearance alone was not a sufficient nor reliable criterion for the sexing of doubtful germ cells.

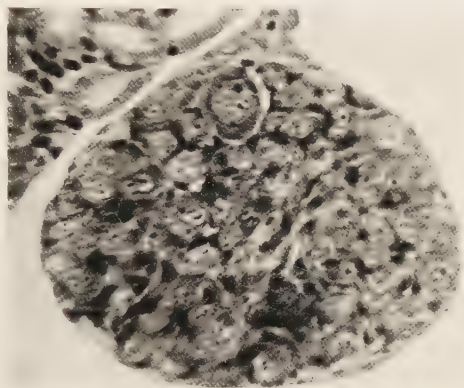


Fig. 10. Tadpole 72 days old. Transection of the right testis, showing an oviform cell near the hilar region.

In a few specimens of this age group, male germ cells, mostly single, have been found entangled in the cortical region. Rarely, in or near the most posterior region of the testes, there still remains a distinct primary genital cavity, surrounding which is a peripheral layer of germ cells. These cells occur either singly or in small cyst-like structures. They possess polymorphic nuclei and are similar, in all their cytological features, to the primary spermatogonia found in the larval testis. The occurrence of such a phenomenon shows that the process of rete growth and of germ cell migration may be retarded for a considerable time in the caudal portion of the developing testis. The germ cells, though still contained in the cortex, have multiplied and developed

germ-cell cysts characteristic of the normal testicular tissue. WITSCHI (1929 a) has advocated that in embryonic testes, germ cells which fail to migrate to the medulla in proper time, transform in the cortical region into oöcytes as in the ovary. In our cases above described, the germ cells have remained in the cortical zone for a long period, as may be judged from the age of the specimen and the developmental stage of the testis. They do not, however, show any transformation into female cells. There is no formation of oöcysts, nor any indication of oögenetic changes, both of which features, as will be elsewhere described, are diagnostic of female sex cells in the larval glands.

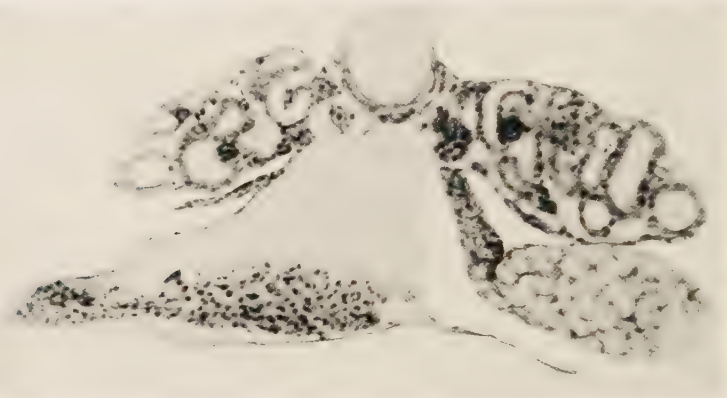


Fig. 11. Tadpole 45 days old and 34 mm. total length. Note the large fat-body on the left side. The right progonad is small and shows no fat-vacuoles. The postcava is turning right toward the liver.

b) *Progonad and postgonad.* In early stages of male development, both progonad and postgonad are composed of stromal mesodermal cells, enclosed externally by a thin peritoneum. Toward their extreme ends, they appear as irregular masses of cells upon the coelomic epithelium. They are short, and their combined length does not exceed the length of the gonad proper of the same side (cases 1 and 2 in table 1). As development proceeds, they increase considerably in length. Table 1 gives the measurements of their relative lengths. It is noted that the postgonad of either side may be longer than that of the opposite side, though in general the left postgonad has a greater length. It is further noted that with a single exception, the left progonad is longer by varying degrees than the right one. The former is invariably larger and much better developed than the latter. This specific difference in growth is due to the fact that the postcava in turning toward the right side of the liver prevents the development of the right progonad (fig. 11). Very rarely the right progonad extends as a small bud of cells beyond

the level of the hepatic flexure of the postcava as in case 8 listed in table I. But here as elsewhere, the right progonad is small and poorly developed, as compared with the progonad of the left side.

Concerning the development of the fat-bodies, the process invariably begins first with the left progonad. The first fat-cells have been found in the left progonad of a male larva of only 30 days in age. The right progonad does not develop any fatty tissue until about 10 days thereafter. The considerable difference in time of appearance of the left and right corpora adiposa is evidently coördinated with the much greater development of the left progonad.

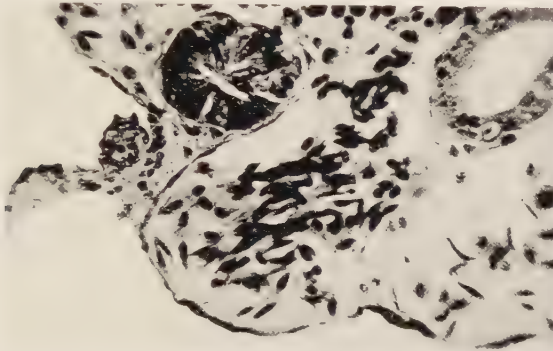


Fig. 12. The oviduct anlage in a metamorphosing male. The large blood vessel nearby is the renal portal vein, above which is the Wolffian duct.

While in later stages both progonads have developed into large fat-bodies, the postgonads remain, throughout the larval development, as small, elongated and rudimentary ligaments (*ligamenta triangulare*), containing scanty loosely arranged stromal tissue. Rarely a small fat body is developed from the postgonad. In some specimens, it appears difficult to determine the boundary between the gonad proper and the postgonad, as the former in these cases merges very gradually into the latter.

Occasional germ cells have been found in the fat-body, and more often in the postgonads. These ectopic germ cells are similar to the normal gonocytes found in the testis, and conform structurally to the same sex type. The occurrence of germ cells in the fat-bodies, postgonads and in other regions outside of the gonad proper, as elsewhere described, is an additional evidence that germ cells are specific in nature and do not transform into other cells no matter where they may happen to become situated.

In the postgonads, many of the ectopic germ cells are seen in the process of expulsion into the visceral cavity. They may be found included in the surface peritoneum, or lying slightly outside of it, or entire-

ly outside, with only a slender attachment to the gonad periphery. The evidence seems to show that the expulsion of ectopic germ cells into the coelom is a normal process of elimination of such cells in the postgonad.

c) *Accessory sex organs.* As previously described in the development of testes, the mesonephric blastema proliferates cells which migrate toward and also into the gonad to form intra- as well as extragonadal rete tissue. This urogenital connection is maintained throughout larval life. The developmental history of the intragonadal rete tissue has been reviewed elsewhere. The extragonadal rete occurs in masses, which are segmentally arranged and are few in number. During metamorphosis, these rete masses begin to develop minute lumina, which are connected with the rete testes, but do not during this period acquire any communication with BIDDER's canal of the mesonephros. These tubules later become developed into functional vasa efferentia.

Toward the completion of metamorphosis, there appear in the region of the testes along the lateral margins of the mesonephroi, distinct thickenings of the peritoneal epithelium (fig. 12). These are the embryonic primordia of MÜLLERIAN ducts. They lie close to, but are distinct from, the WOLFFIAN ducts and the renal portal veins. The cells composing them are ordinary peritoneal elements, which are formed by cell proliferation *in situ*, and from adjacent parts of the kidney peritoneum. The cellular cords show no definite organization nor any lumina.

d) *Secondary sex characters.* In metamorphosing males, there are developed minute papillae on the dorsal surface of the crura, which bear a superficial resemblance to the rut warts found abundantly in the adult females. Throughout the larval development, the tadpoles show no external structures or characters, which may be of significance in sexing or in sex recognition.

B. Female Development.

a) *Gonad proper (OVARY).* Gross structure: It is difficult to distinguish between young ovaries and testes by macroscopical examination. They are similar to each other in position of attachment and in general shape, but the ovary is generally slightly larger in size and does not show any segmental appearance (fig. 13). In further development, the ovaries enlarge rapidly and become folded and wrinkled, owing to the formation of ovarian sacs and to inequalities in the growth of the contained oöcytes (fig. 14). Table 2 shows the relative lengths of the two ovaries and their length increase throughout the larval development. The left ovary is generally longer than the right one, and also extends further cranially. With the approach of metamorphosis, the ovaries become much longer and larger than testes of similar age, and can be readily distinguished from the latter.

Table 2. *Length Measurements of Female Gonads.*

Specimens			Length of gonads in mm.					
Age in days	Length in mm.		Progonads (Fat-bodies)		Gonads proper (Ovaries)		Postgonads	
			Lt	Rt	Lt	Rt	Lt	Rt
1.	28	20	0,36	0,22	0,51	0,39	0,16	0,23
2.	32	24	0,44	0,32	0,58	0,58	0,26	0,26
3.	37	28	0,34	0,16	0,58	0,45	0,31	0,23
4.	38	34	0,80	0,30	0,76	0,86	0,21	0,31
5.	43	38	0,80	0,72	0,63	0,75	0,47	0,45
6.	51	35	0,60	0,48	1,13	0,87	0,18	0,29
7.	51	39	0,91	0,76	1,20	1,15	0,13	0,25
8.	59	45	—	—	0,85	0,98	0,15	0,05
9.	66	51	—	—	1,33	0,86	0,16	0,23
10.	66	Metamorphosing	0,95	0,80	0,90	0,76	0,16	0,11
11.	72	„	—	—	1,22	0,60	0,13	0,07
12.	72	„	—	—	1,64	1,28	0,30	0,30

Microscopical structure: For convenience of description, the materials may be presented in stages as follows:

1. *Larva 28 days old and 20 mm. total length.* In essential structure, both gonads closely resemble those in the early stage of testis development. The germ cells, however, instead of migrating inward to the rete cord



Fig. 13. Ovaries in a tadpole 55 days old and 30 mm. long.



Fig. 14. Larval ovaries near the time of metamorphosis.

as in the case of prospective testis, remain in their original position, thereby forming a distinct cortical germinal epithelium, which later develops into the definitive ovarian cortex.

The gonocytes are large in size and are characterized, as those in the young testis, by large polymorphic nuclei and many brownish granules in the otherwise clear, homogeneous and faintly-stained protoplasm. An intranuclear framework of chromatin is distinct, and upon it are suspended a number of spherical nucleoli staining intensely black. In size, the nucleoli range from 2 to 1 micron or less. They are generally large, when present in small numbers, and smaller, when found in larger numbers. With successive divisions, the gonocytes or primary oögonia,

as they may now be called, give rise to two different types of cells. One type conforms to the structure of the parent cell, while the other is smaller, with a proportionally smaller nucleus which is either oval in shape or slightly notched. Cells of the latter type are only few in number in this specimen. They are secondary oögonia, as clearly shown by their derivation and particularly by their future history. The formation of these gonial cells during the early larval period is the first morphological evidence of the female sex. In case of the male, secondary spermatogonia do not develop, as will be shown later, until after metamorphosis. The secondary oögonia, as soon as formed, are found to remain in groups, which tendency leads to the formation of the so-called "egg nests" or oöcysts, characteristic of the early ovary development.

2. *Larvae 30—35 days old and 25—35 mm. total length.* With continued thickening of the germinal epithelium, the ovaries increase considerably in diameter. Definitive oöcysts become prominent structures in the gonad, mostly massed along its distal periphery. They contain only a few secondary oögonia, surrounded by cellular follicles. Many of the oöcysts encountered in later stages are distinctly larger, containing as many as 8 or 10 cells. The secondary oögonia during this period are characterized, in contrast to the primary gonia of either sex, by the small size of the cell body and the rounded form and compact structure of the nucleus. They contain one or two or rarely three large, and sometimes a few smaller nucleoli suspended on a distinct chromatin reticulum, and show diminishing amounts of pigment granules in their protoplasm. Cells contained in the same oöcyst remain delimited from one another by thin plasma membranes. There is no syncytial formation by these cells, nor any fusion of their nuclei. All secondary oögonia are formed by division from primary oögonia or from preexisting secondary oögonia. There is no evidence of their formation from somatic tissues of the ovary, nor of their transformation into follicular or granulosa cells, as claimed by HOFFMANN (1886), NUSSBAUM (1880), GEMMILL (1896), and others.

As soon as an oöcyst is fully developed, or soon thereafter, the secondary oögonia enter maturation changes. The oöcyte, when first formed, is characterized by the presence of a conspicuous intranuclear chromatic network, composed of numerous, much coiled fine filaments, on which are scattered many isolated irregular chromatin clumps of various sizes, and one or sometimes a few large and rounded nucleoli. This reticulum soon becomes more distinct and gradually assumes the appearance of well-defined, yet delicate threads in spireme formation. These leptotene threads have a beaded appearance, showing irregular thickenings throughout at more or less regular intervals. They soon undergo a gradual process of condensation and contraction toward one side of the nuclear space, forming eventually a dense deeply-stained

and more or less rounded mass, often called the contraction figure or synzesis mass. Even in such a condensed condition, the chromatin threads remain recognizable, and short filaments are seen radiating outward from the concentrated mass. The nucleolus, usually only one during this period, is generally found enclosed by, and is sometimes entirely concealed among the much tangled and darkly stained threads. The cytoplasm of the synizetic oöcytes shows slight increase in volume, and contains one or a few scattered granular bodies, which stain black and bear some resemblance to the pigment granules found abundantly in early germ cells. Synzesis in normal oögenesis appears to be of wide occurrence in the vertebrates (see for example, VON WINIWARTER and SAINMONT, 1908; KING 1908; OKKELBERG 1921; HANN 1927; GOLDSMITH 1928; and SWEZY 1929). BOUIN (1901) among others have reported degeneration of oöcytes during this phase of oögenesis. KING (1908) has pointed out that the cells considered as degenerating are probably oöcytes undergoing synzesis.

The chromatin content of the synizetic mass soon resolves itself into thick spiremes, which, on account of their mode of origin, are in the form of irregular loops radiating from one side of the nucleus, an appearance which naturally suggests a pachytene bouquet. This formation later disappears as the spiremes become evenly distributed throughout the interior of the nucleus. Generally one or rarely more, large, spherical nucleoli are seen, lying near, or closely applied to the nuclear membrane and are usually, though not always, associated with the framework of spiremes. Minute dark granules are often found dispersed along the nuclear periphery and sometimes surrounding the nucleoli. In early pachytene, the nucleus measures about 14 micra in diameter, and the cell body from 20 to 25 micra, both larger than those of earlier oöcytes.

All maturation cells of this period occur in oöcysts surrounded by thin and irregular cellular follicles. They are found in the various stages of the synaptic period or the period of first growth¹, representing leptotene, synzesis and pachytene stages. Oöcytes of the same oöcysts are not always found in the same stage of maturation.

Scattered among the oöcysts and especially in the proximal region of the ovary, there is found a number of primary oögonia (fig. 15) readily recognizable by their large size, nuclear polymorphism and characteristic staining reaction. These primary gonía perpetuate themselves throughout the larval period, and constantly give rise to secondary oögonia, which subsequently enter maturation to become oöcytes.

¹ Maturation stages during the synaptic phases or the synaptic period have been referred to as "pseudoreduction stages" (WITSCHI, SWINGLE), "pre-reduction stages" (WITSCHI), or "prematurations stages" (CHRISTENSEN), all of which terms are unsatisfactory, and are, in many respects, misleading.

All female germ cells, or practically all of them, whether in multiplication or in maturation, whether singly or in cysts, are grouped along the gonad periphery, forming a distinct ovarian cortex. The primary genital cavity remains distinct in the medullary portion with the rete cords segmentally arranged throughout. Occasional germ cells have been found lying amid the loose stromal tissue of this cavity, that is, in the medullary portion between the rete cords, but not within the



Fig. 15. Tadpole 48 days old and 35 mm. total length. Transection of the ovary, showing oöcysts and oöcytes in various stages of oögenesis. Note the primary oögonia near the mesovarial attachment and among the developing oöcysts.

cords. It is a significant fact that these germ cells, though not included in the ovarian cortex, give rise by division to typical oöcysts (fig. 16), the cells of which subsequently undergo oögenesis like the cortical germ cells. Figure 17 and 18 show in transection and longisection respectively, the maturing oöcysts in the inter-cordal medulla. These oöcysts continue to develop and remain in their position for a long time. Eventually with the obliteration of the primary genital cavity, they become incorporated with the ovarian cortex.

Expulsion of germ cells has been rarely encountered. When found, the expelled elements may be gonial cells or oöcytes, normal or rarely degenerating. Occasionally they are seen in the visceral cavity, lying entirely outside of, but adjacent to the ovary.

As in testis development, ectopic germ cells or groups of germ cells have been found in the mesenchyme at the base of the dorsal mesentery, in the mesovarium, and in regions between them. These isolated cells or cell-groups are evidently left there owing to their failure to reach the gonadogenic region during the early migration of the germ cells. They remain undeveloped, and eventually disappear either by degenerative changes or possibly by expulsion into the body cavity. Some of them may, however, persist and develop into accessory ovaries. The fact that in no case are they found to have transformed into somatic cells or into cells similar to those among which they lie, is a further evidence



Fig. 16. Tadpole 32 days old and 24 mm. total length. Arrow shows an oöcyst of secondary oögonia in the intercordal medulla.

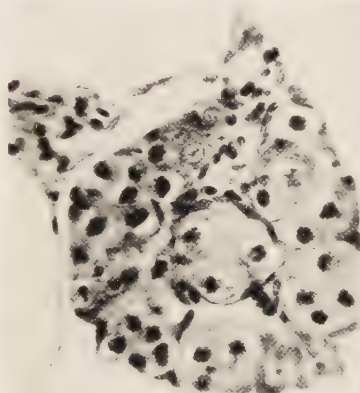


Fig. 17. Tadpole 32 days old and 27 mm. total length. Note oöcytes in the medullary oöcyst.

that germ cells are specific in nature morphologically as well as genetically.

By a rearrangement of the component cells, the rete cords soon acquire small lumina, one or more in each cord. These lumina appear first in, and are, during this period, confined to, the distal tips of the rete cords. The connection of the rete tissue with the nephrogenous stroma is distinct, with masses of cells derived from the mesonephroi accumulating in the mesovarium.

3. *Late stages of ovary development.* The pachytene cells now enter the second growth period, during which they undergo further growth in size, showing enlargements of both nucleus and cell body. The oöplasm assumes a granular appearance, staining more or less bluish with iron hematoxylin, and the nuclear spiremes become simultaneously attenuated and appear as isolated oxyphilous strands of chromatin, loose in texture and in outline. Many nuclear bodies are developed, which stain deeply and are of various sizes and shapes. They are scattered about the nuclear

space, and generally aggregated most densely along the periphery. The cytoplasmic granules show up distinctly as dark and more or less rounded bodies.

The oöcytes of the second growth period as above described, may be called ova¹, owing to their resemblance to the mature egg-cell. The



Fig. 18. Tadpole 33 days old. A longisecton of a young ovary, showing 4 rete cords in the medullary portion, and oöcytes in the cortex as well as in the inter-cordal medulla.

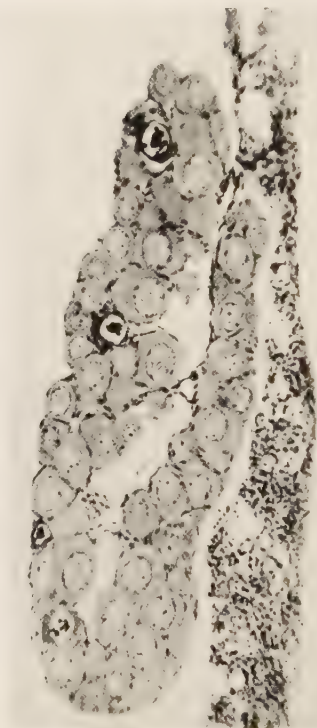


Fig. 19. Tadpole 64 days old. In the ovarian cortex there are contained many growing ova, a few of which are in the process of degeneration. The ovarian sacs are distinct. Compare with fig. 3.

wall of the oöcyst is soon ruptured, and follicle cells develop around individual ova to form the membrana granulosa. The stroma cells are scanty, and some of them become flattened against the growing oöcytes, forming the primordia of the theca folliculi.

While the growth of oöcytes is under way, the lumina of the rete cord enlarge rapidly and become confluent into large cavities lined by

¹ WITSCHI has used the term "auxocyte" to denote the primary oöcyte in the second growth period. This is a wrong usage, because the term "auxocyte" is used to include any cytes (spermatocytes or oöcytes) during the entire growth period (WILSON 1925).

thin mesothelia of rete cells. There is generally only one such cavity in a rete cord, which becomes now known as an ovarian sac, homologous to the rete testis in the male gonad. In further development, the ovarian sacs expand, eventually obliterating the primary genital cavity. The sacs are separated from one another by thin mesothelial partitions (fig. 19), which become later transformed into the septa of the adult ovary.

Fully developed larval ovaries show numerous growing ova of various sizes in the deeper region of the cortex, often protruding into, and sometimes entirely obliterating, the central ovarian cavity. Primary oögonia, either massed or singly, and cysts of secondary oögonia or of oöcytes in various stages of synapsis are confined to the periphery of the gonad.

Throughout larval oögenesis, and especially during the period of second growth, degenerating germ cells have been sporadically encountered (fig. 19). The degenerative process is probably induced by insufficient nutritive supply. The nuclear content of the degenerating oöcytes becomes clumped into homogeneous intensely-staining basophilic granules or bodies of various sizes and peculiar shapes, which may be scattered throughout or massed together without any definite organization. The oöplasm in the meantime undergoes reduction in quantity and stains more deeply than formerly. The whole structure is abnormal and is evidently undergoing gradual resorption eventually leading to complete degeneration. Germ cells, whether normal or degenerating, remain distinct from the surrounding somatic elements. Degenerating cells are few as compared with the total number of germ cells present.

b) *Progonad and postgonad*. In structure and development, the progonads of the female are similar to those in males. The left fat-body appears earlier, and is always better developed, than the right one. Our evidence is in accord with the results of many previous investigators (e. g. VON WITTICH 1853; MARSHALL and BLES 1890; KUSCHAKEWITSCH, 1910; and McCOSH 1928, 1930) that the corpus adiposum in either sex is directly derived from the anterior portion of the germ gland, or the progonad, and not from cells which have secondarily come into connection with the anterior end of the germinal ridge, as KING (1908) believes. GILES (1890) claims that the fat-body is derived from the pronephros or the head-kidney, and GIGLIO-TOS (1895) that it develops from the connective tissue of the adventitia of the postcava. Neither claim is correct as seen from our present data.

The postgonads in the female are rudimentary in structure as those in the male, and are, in later stages of development, very much shorter than the latter (Compare tables 1 and 2). The reduction in length of the postgonad in the female sex is apparently correlated with the expansion of the ovary due to the growth of the contained oöcytes. The

postgonad persists in the adult female as ligamentum triangulare. Rarely it develops into a small adipose body (cf. PATZELTS adult specimen 1918).

Ectopic germ cells found in the postgonad generally disappear through expulsion into the coelom. In a specimen, a small mass of germ cells is found in the postgonad about 0.1 mm. posterior to the ovary. It is considered possible that such an ectopic cell-group may in time develop into an accessory or heterotopic germ gland. In a single instance, two oöcytes are seen in the fat-body, lying along the surface peritoneum near the attachment of the mesovarium.

c) *Accessory sex organs.* The rete cords in the female, unlike those in the male, are abortive structures. The intragonadal cords develop into ovarian sacs which are of no functional importance, while the extra-gonadal cords, during early stages, appear in the mesovarium as irregular masses of cells, which remain rudimentary in later development, without forming any tubules corresponding to the vasa efferentia in the male.

The oviduct anlagen are developed during metamorphosis. They are armed, as in the male, by cell proliferation from the peritoneum along

Table 3. *Comparison and Contrast of the Development of Ovary and Testis During the Larval Period.*

Ovary	Testis
Indifferent gonad with primordial rete cords in the medulla and a germinal layer at the cortex.	Indifferent gonad with primordial rete cords in the medulla and a germinal layer at the cortex.
Germ cells generally remain in the peripheral germinal epithelium, or occasionally migrate to the inter-cordal medulla.	Germ cells migrate into the rete cords, which have in the meanwhile enlarged and become anastomosed with one another to form a medullary germinal core.
Formation of secondary oögonia and their grouping into oöcysts.	Multiplication of germ cells as primary spermatogonia.
Occurrence of maturation (or oögenesis): Germ cells become oöcytes. They undergo synaptic changes (first growth), following which they enter the second growth period, forming typical ova. Disintegration of oöcysts with the growth of the contained ova.	Absence of maturation phenomena: Germ cells remain in primary multiplication.
Development of the definitive ovarian cortex.	Formation of testicular ampullae, the future seminiferous tubules.
Formation of secondary genital cavity: Ovarial sacs (rete ovarii).	Formation of secondary genital cavity: Rete efferentia (rete testis).
No transformation of mesodermal cells into germ cells or <i>vice versa</i> .	No transformation of mesodermal cells into germ cells or <i>vice versa</i> .
Occasional degeneration and expulsion of germ cells.	Occasional degeneration and expulsion of germ cells.
Ovary becomes large and elongate, with an irregular and much corrugated outline.	Testis remains small, compact and narrow, with a fairly regular and smooth contour.

the lateral sides of the kidneys. Toward the completion of metamorphosis, they are distinct structures composed of loose tissue, with a layer of epithelial cells surrounding the tiny central lumen.

d) *Secondary sex characters*. During metamorphosis, dermal papillae are developed on the upper surface of the shanks. They appear similar to those found in metamorphosing males. The female tadpoles like those of the male sex show no external characters which may be used in sex identification.

C. General Consideration.

a) *Differentiation of sex*. The problem of sex differentiation in the frog has been investigated in greater or less detail by various writers of the past century (von WITTICH 1853; NUSSBAUM 1880; HOFFMANN 1886; SEMON 1891; GEMMILL 1896). The literature covering this subject has been reviewed and discussed by KUSCHAKEWITSCH (1910) and WITSCHI (1914). BOTIN (1901), in his work on *Rana temporaria*, has arrived at certain important principles, which may best be summarized in his own words:

„Nous avons succesivement constaté: dans le premier cas (male), des cordons génitaux importants, un épithélium germinatif moins développé que dans le deuxième cas (female), de nombreux ovules primordiaux présentant des phénomènes de clivage et de bourgeonnement; dans le second cas, des cordons génitaux moyennement développés, un épithélium germinatif bien développé, des ovules primordiaux en activité cinétique et des nids d'ovules primordiaux.“

These results have been supported by subsequent researches, and have been adopted by recent investigators as diagnostic factors of sex. KUSCHAKEWITSCH (1910) claims that „Ein Tier ist als männlichen Geschlechtes zu bezeichnen, sobald seine Genitalstränge (rete cords) sich in bezug auf die Keimzellenbildung als produktive erweisen“. WITSCHI (1914), and subsequently SWINGLE (1926) have further emphasized the important rôle of rete cords in the process of sex differentiation. In the prospective male, the rete cords grow rapidly and the germ cells migrate inward to the cords where they continue in active multiplication. In the female, on the other hand, the development of rete cords is early arrested and the germ cells remain in the thickened germinal epithelium and most of them soon begin maturation changes and become oöcytes.

In *Rana cantabrigensis*, the early developmental processes involved in the male differentiation confirm in essential points the view of KUSCHAKEWITSCH and WITSCHI. Our evidence, however, contradicts KUSCHAKEWITSCH's interpretation that the germ cells originate from among the rete cells, and is in accord with the results of WITSCHI and others that they migrate to the rete medulla from the gonadic cortex. The migrating germ cells accompanied by their follicles, do not, as seen in our description of the process, occur in definite cords nor in distinct groups, but are generally isolated from one another or only loosely

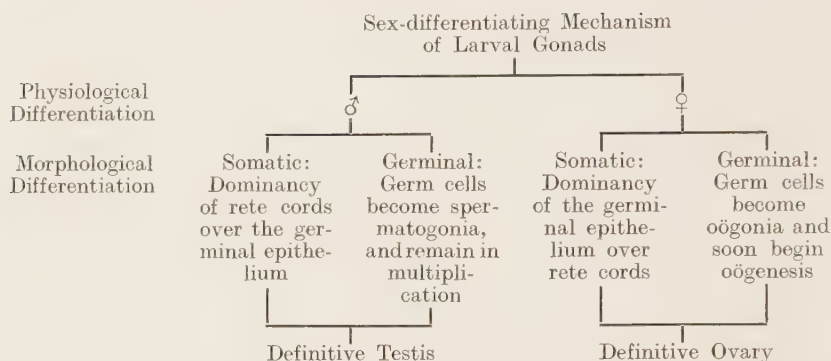
associated. Thus, in our frog material, there are apparently no sex cords to speak of.

In the female development, the germ cells remain in the peripheral germinal epithelium as primary oögonia, the majority of which, however, soon become transformed into secondary oögonia, and subsequently grouped in oöcysts. The formation of secondary oögonia is, therefore, one of the most important early sex criteria as regards the germ cells. (HAMPTON (1913) has noted that in *Rana temporaria*, *Bufo*, and *Hyla*, if the gonocytes retain their characteristically polymorphic nuclei and remain isolated, the germ gland is destined to form a testes, and if the germ cells become grouped in follicles and their nuclei lose the polymorphic form and become round and clear, an ovary will result. This histological fact becomes more significant, when viewed from an embryological standpoint. The large sex cells with polymorphic nuclei are primary gonia no matter in which sex they may occur. In the young ovary, the primary oögonia early produce by repeated division many secondary oögonia in characteristic cyst formation, while in the testis, the germ cells remain in the primary gonial stage, as already described, throughout the larval period, and do not produce secondary spermatogonia until after metamorphosis. It is, therefore, the marked difference in the time of development of secondary gonia in the respective sexes, which is considered of significance in sex diagnosis.

With the progress of sex differentiation, ovaries and testes become readily distinguishable not merely by the characteristics of the germ cells contained in them, but also by the condition of the somatic components as well as of the gonad as a whole. Table 3 gives a summary review, from a comparative standpoint, of the various structures formed, and the various important processes involved in the development of larval gonads. In the testis, it may be noted, the rete medulla becomes developed as a prominent germinal structure, surrounded on its free surface by a scanty peripheral connectivum which, with the investing peritoneum, may be homologized with the ovarian cortex. In the female, the rete tissue undergoes abortive changes, forming ovarian sacs which remain rudimentary throughout life. The germ cells of the testis become nested in cysts, which structures are, however, in no way, comparable to the oöcysts in the ovary. The testicular cysts consist of primary spermatogonia, while the oöcysts are composed of secondary oögonia and in later stages, oöcytes. The former structures are the embryonic fundamentals of the seminiferous tubules, while the latter eventually disappear when the contained oöcytes begin their second growth. The true testicular homologue of the oöcysts are spermatocysts, which are, as will be described in the postlarval development of the testis, developed within the seminiferous tubules. This fact has led many writers to homologize the testis tubules with the germinal cortex of the ovary.

The origin of the somatic components of the seminiferous tubules may appropriately be considered here. WITSCHI (1929 b) has maintained that the seminiferous tubules are derived from the rete blastema, without any participation of the germinal epithelium or proliferations from it. It may be noted, however, that the male germ cells in migrating to the rete tissue, are invariably accompanied by their follicles, which are, as shown by our evidence (CHENG 1932), of a peritoneal origin. The future history of the follicle cells remains obscured. Some of them may remain as such throughout the development, while others become apparently developed into the covering epithelia of the testis tubules, or at least form a greater or smaller part of such.

WITSCHI, in his theory of localized sex-differentiators („*lokalisierte Innenfaktoren*“) claims that the peritoneum of the gonad is sexually indifferent, and contains undifferentiated or indifferent germ cells. The rete cords contain the male-differentiating system (M), which, when active or activated, “causes the germ cells to migrate from the cortex into the medulla of the gonad and to transform into spermatogonia”. The cortex, or as he calls it, the deeper layers of the germinal epithelium, include the female-differentiating system (F), “which causes germ cells to differentiate into oöcytes and inhibits the growth of the rete cords”. The localization theory, which WITSCHI has maintained in many of his papers, and which he considers as firmly established, needs careful consideration in the light of our new findings. In the testis development of our frogs, cases have been encountered, in which the germ cells are belated in the process of inward migration, and get stranded in the cortical portion. Such cells do not, however, become transformed into oöcytes as one would expect from WITSCHI's hypothesis. They may remain single or divide to form germ cell cysts similar in every respect to those found in the rete medulla. Likewise in early ovary development occasional oögonia or cysts of such cells become transported to the medullary portion in between the rete cords, thus, to a region which may be designated as inter-cordal medulla in contradistinction to the rete medulla. These female cells, though not contained in the ovarian cortex, enter oögenesis in a normal manner, and give rise to typical oöcytes. They may remain in the inter-cordal medulla for varying lengths of time, and may even become secondarily attached to the rete cords. Eventually, as the rete cords degenerate, they are incorporated into the definitive ovarian cortex. These facts seem to indicate that the morphogenetic mechanism of sex differentiation can hardly be regarded as being strictly localized. The various zones developed and the structures formed in the differentiating gonad are merely the morphological expression of the fundamental embryonic physiological processes of sex differentiation, and should not be interpreted as a localization of sex tendencies, or of sex-specific morphogenetic substances. The idea may be represented in a scheme as follows:



WITSCHI has also claimed that the surface peritoneum of the gonad is indifferent and that "even the gonia of the young and the adult ovary, though called oögonia, stay undetermined as long as they are embedded in the peritoneum". In other words, the indifferent gonia, according to WITSCHI, are identified as such and can only be so identified by the position which they occupy in the gonad. This is a misleading interpretation, and is, in many respects, histologically unjustifiable. Throughout our description of the developing ovary, we have noted and emphasized the presence and the developmental activities of the primary oögonia. Morphologically, these cells appear to be identical with the primary gonia of the male sex. Experimental masculation of larval ovaries (BURNS 1924—1930; WITSCHI 1929 *b*; HUMPHREY 1929—1930; PRIQUET 1930) is strongly indicative of the possibility that these primary oögonia, normally giving rise to secondary oögonia and oöcytes, may, under certain conditions, change their normal course of development and become differentiated along the male direction. The sex condition of the primary oögonia is apparently such that they are capable of developing into either sex, and the direction of their differentiation is determined by the balance assumed and maintained by the sex-differentiating complex in whatever way such a balance may be adjusted. It is apparently this developmental capacity of the primary oögonia that renders possible the gonadic transformation of sex. All available evidence, morphological as well as experimental, when considered with reference to each other, seems to support our assumption that the primary oögonia are potentially equipped for and are capable of developing, under different conditions, into germ cells of either sex. They may, therefore, be regarded as sexually indifferent or undifferentiated. These indifferent gonia are not always embedded in the gonad peritoneum, as advocated by the localization theory. They may be found near the surface, or even deeper among the developing oöcysts in almost any portion of the cortex. In whatever region they may occur, these

are morphologically of the same type and there is no evidence that they are physiologically or sexually different. The position which these gonial cells occupy in the ovary is, therefore, a matter of little significance: their most important characteristics are the cytological features which actually identify them as such.

In larval testes, all germ cells remain as primary spermatogonia, which correspond, in stage of development, to the primary oögonia found in the ovary. The works of CHAMPY (1913), and ROXAS (1929) appear to show that the sex cells of the frog testis may occasionally become transformed into oöcytes or ovum-like cells. MEYNS (1912), in transplantation experiments with frogs, has found female cells in testicular grafts undergoing regeneration. Similar and even more striking evidences are available in other amphibians (GUÉYNOT and PONSE 1923; DU BOIS and BEAUMONT 1927; DU BOIS 1927; WELTI 1928; and BEAUMONT 1929). CHAMPY (1921), working on tritons, and more recently BURNS (1924—1930) on *Ambystoma*, have shown, by various methods, a gonadic transformation of sex from male to female. All these results seem explicable on the assumption that the primary spermatogonia are sexually similar to the primary oögonia, and are likewise capable of differentiating into either sex. In both male and female, the bisexual potentiality of germ cells is apparently lost or at least suppressed, as the primary gonia develop into secondary gonia and become increasingly specialized in further differentiation. In our intersexual materials previously described (CHENG 1930), oöcytes, scattered or massed, have been found amid the growing testicular tissues. Despite their abnormal position, these oöcytes remain as such and may even continue to develop as such, without showing any sign of transformation to the opposite sex. This is an additional evidence that germ cells can not be altered in sex, after they are once definitely differentiated.

In the larval development of both sexes, expulsion of germ cells has been occasionally encountered. BOUIN (1900, 1901) reports that he has observed in tadpoles of *Rana temporaria* expulsion of a great number of degenerating gonocytes (*ponte d'ovules primordiaux*), resulting in a considerable numerical reduction of these cells. He further claims that there is a relative frequency of this process in different sexes. KUSCHAKEWITSCH (1910) has observed germ-cell expulsion in *Rana esculenta*, but no sex difference in the extent of this process. DUSTIN (1907) and WITSCHI (1914), working on the same species which BOUIN used for his studies, have found no evidence of «*ponte d'ovules primordiaux*» in normal tadpoles. WITSCHI observed this process „*bei ausmetamorphosierten Kälte- und Hitzetieren (Weibchen oder Umwandlungsformen)*“. In *Rana cantabrigensis*, germ cells are occasionally observed in the actual process of being shed bodily from the gonad into the visceral cavity. The extreme rarity of such cases shows that this process is but an embryo-

logical abnormality of germ cells and plays no rôle in the normal development of gonads or of sex in our species. In the postgonads, expulsion of germ cells appear to be of frequent occurrence. Here the expelled elements are ectopic germ cells which are not concerned in the process of sex differentiation.

From the foregoing account, it is obvious that our frog material belongs to the so-called differentiated type of sex development. PFLÜGER early demonstrated (1882) the existence of differentiated and undifferentiated local strains in the same species of frogs. Subsequent investigations especially by KUSCHAKEWITSCH, WITSCHI and SWINGLE not only confirm this, but further show that there are different types of sex differentiation and gonad development in the undifferentiated frog race. It remains problematical whether or not such different local strains may also occur in *Rana cantabrigensis*, an interesting problem which merits further investigation.

b) *Continuity of germ Cells.* KOLESSNIKOW (1878), HOFFMANN (1886), BOUIN (1901), DUSTIN (1907), and KUSCHAKEWITSCH (1910) have claimed for the various species of European frogs a germinal epithelial origin of germ cells. Bouin and subsequent writers as named above have described the development of two generations of gonocytes. The first generation may be derived from the vitelline entoderm (KUSCHAKEWITSCH), or from mesenchymal and peritoneal elements (BOUIN, DUSTIN), while the second generation is mostly proliferated from the gonad peritoneum, which is accordingly designated and described as the "germinal epithelium". SWINGLE has reported abortive prespermatogenesis in the bullfrog tadpoles, as a result of which process nearly all cells of the primary germ line degenerate. A second line of germ cells appear, which, according to his interpretation (1926), are derived from the lineal descendants of the primordial germ cells.

In *Rana cantabrigensis*, the germ cells are readily distinguishable from the mesodermal elements surrounding them in any stage of the larval period. They are genetically continuous and can be traced throughout every phase of development. Prespermatogenesis does not take place in the numerous tadpoles examined, and the larval oögenesis is in every respect normal, producing oöcytes which later develop into functional eggs. In either sex, there is no widespread degeneration or expulsion of germ cells which might be taken as indicative of a possible regeneration or neoformation of germ cells from a somatic source, as claimed by BOUIN among others. All germ cells, whether in testes or in ovaries, are direct descendants of the primordial germ cells which have a definite origin from outside the gonadogenic region, and which show a specific genesis during early ontogenetic development.

II. Postlarval Period.

A. Male Development.

1. From the completion of metamorphosis to the end of the first year¹. Frogs during this period measure from 16 to 26 mm. in body length.

In newly metamorphosed frogs, the testis tubules are more or less regularly arranged along the surface of the gonad, and are separated one from another by their thin investing epithelia. Stromal tissue is scanty, and mostly grouped in the center of the testis. Rete tubules can be traced here and there, though they are sometimes obscured by the crowding of adjacent structures. With the progress of development, the seminiferous tubules begin to branch and continue to increase in size and length, as a result of which, they become convoluted and coiled in various ways. Thus, their original orientation and more or less symmetrical arrangement are entirely lost, and the gonad appears in a transection as a mass of many tubules separated by only sparse intertubular tissue.

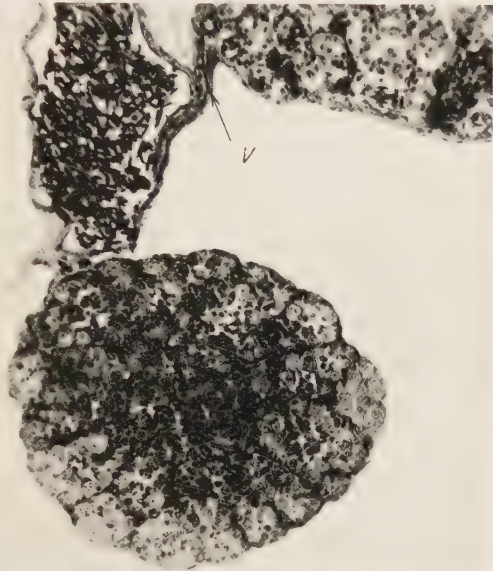


Fig. 20. Transection of the left testis shown in figure 22. Note the numerous secondary spermatogonia in the seminiferous tubules. Among them are found larger cells which are primary spermatogonia. In the mesorchium, a vas efferens (v) can be seen in close contact with Bidder's canal.

Correlated with the enlargement and elongation of testis tubules, the contained germ cells continue to multiply rapidly. The cells thus produced often become so numerous as to obliterate the lumina of the tubules. The primary spermatogonia in division give rise either to more primary gonidia, or to secondary spermatogonia. The latter elements, as soon as formed, undergo further division, and produce more of their kind, all of which are then grouped in spermatogonial cysts or spermatocysts. Eventually, thus, the tubules are filled with many cysts of second-

¹ Age is calculated from the time of egg-laying, which generally occurs during late March and early April.

dary spermatogonia, and with relatively few primary gonial cells dispersed throughout (fig. 20). Degenerating gonial cells either singly or in cysts have been seen in otherwise normal testicular tissue. They appear as agglomerations of pyknotic cellular debris, undergoing gradual resorption.

The primary gonial cells during this time measure 18—25 micra in diameter, and are characterized, as hitherto, by their large polymorphic nuclei and a faintly staining nuclear network with basophilic nucleoli scattered on it. The nucleoli are generally few and large, and are rounded in form, though often assuming a slightly irregular contour owing to the concentration of chromatin granules around them. The cytoplasm is homogeneous and finely granular. It contains, in some cases, one or a few small dark granules, which bear some resemblance to the pigment granules found in germ cells of earlier testes, and are probably the remains of such. The secondary spermatogonia are small, usually only about half the size of the primary spermatogonia, and contain proportionally smaller nuclei, which are compact in structure and are generally ovoid in shape, though sometimes slightly lobulated or notched. The nucleoli range in number from few to more than ten, and are smaller than most of the nucleoli found in primary gonial cells. The intranuclear reticulum stains quite deeply, with numerous masses of chromatin in evidence.

The development of two kinds of gonial cells here is an important embryological fact in the history of male germ cells. The secondary spermatogonia later undergo maturation to produce spermatozoa for spawning, while a sufficient number of primary spermatogonia is always reserved to furnish cells for subsequent spermatogeneses throughout the whole period of functional life of the testis.

The process of germ cell multiplication above described is characteristic of testis development during this period. In a small number of specimens, however, some of the germ cells are seen to have undergone maturation, a phenomenon, which, for reasons considered below, is to be designated as prespermatogenesis in (HAMPTON'S) terminology (1913). The processes involved in this maturation cycle appear, as far as morphological evidences are concerned, to be normal in every respect, and the maturation cells are found in various stages of development leading from early spermatocytes to mature spermatozoa. These cells may occur only in a few tubules found sporadically throughout the gonad, or in nearly every tubule, but in small numbers disposed among the numerous multiplying germ cells. The paucity of the maturing and mature germ cells is in itself an indication of the precocious nature of this phenomenon. In adult testes of the same season, spermatogenesis is well advanced and mature spermatozoa are abundant in the seminiferous tubules. It should be further noted that most of the juvenile testes which show mature sperms still lack tubule connections (vasa

efferentia) with BIDDER's canal of the mesonephros, and the frogs in question are small and develop no nuptial pads nor any other secondary sex characters of the male. Taking all these facts into consideration, it becomes obvious that the maturation of male germ cells during this stage of development is a precocious feature, and that the animals do not function sexually, despite the presence of spermatozoa in their testes.

Externally the young postlarval testes appear somewhat oblong, with a broad end in front, while tapering posteriorly into the small



Fig. 21. Testes and MÜLLERIAN ducts of a newly metamorphosed male frog. Specimen collected on July 16th, measuring 20 mm. in body length.



Fig. 22. Testes, MÜLLERIAN ducts and seminal vesicles of a winter frog (collected December 29th) 24 mm. in body length, about the size of a full-grown first-year male. The MÜLLERIAN ducts are degenerating.

postgonad (fig. 21). They later become oval-shaped (fig. 22), assuming the characteristic form of the adult testis. Rudimentary postgonads are found even in adult males.

MÜLLERIAN ducts remain vestigial in structure. At the height of their development, they appear, upon external examination, as fine cords running along the lateral side of the kidneys, and extending anteriorly to the level of the fore limbs. They are nearly straight, and show no convolutions characteristic of the oviduct in adult females. Microscopically they are seen as small projecting ridges from the parietal peritoneum, and are composed of loose tissue, the cells of which are occasionally arranged in the form of a potential tubule without any actual lumen. Behind the kidneys, the cellular ridges flatten down as mere thickenings of the general peritoneal lining, and gradually dis-

appear. Throughout the entire length, the oviduct anlagen remain independent of the WOLFFian ducts, with which they are closely associated.

Toward the end of the first year, the MÜLLERIAN ridges have become greatly reduced both in size and in length. In many specimens they are identifiable only along the posterior portion of the kidneys as irregular strands of cells. The extent of their degeneration appears to be conditioned by the degree of body development as indicated by length. In specimens showing prespermatogenesis, the oviduct anlagen remain distinct as in normal males.

After metamorphosis the vasa efferentia continue to develop, and in frogs collected in October and December, they are found to have grown to the median portion of the mesonephroi, and acquire lumen connections with BIDDER's canal (fig. 20). In younger specimens no such connections have been observed. KUSCHAKEWITSCH (1910) figured the vas efferens of a two-year-old male frog (*Rana esculenta*), which still lacked definite connection with the kidney tubule. HOSKINS and HOSKINS (1919) found normal vasa efferentia developed in thyroidless tadpoles of *Rana sylvatica*, killed about a year after the normal time of metamorphosis.

In young frogs of about 16—18 mm. in body length, seminal vesicles appear as tubular buds from the posterior part of the WOLFFian duct. Their subsequent development consists of further budding, enlargement and branching of these diverticula. Macroscopically, the primordial seminal vesicles appear as masses of glandular swellings symmetrically placed along the lateral side of the WOLFFian ducts. The lumina of the vesicles remain at all times connected with the WOLFFian ducts of the respective side.

Many of the newly metamorphosed frogs show few scattered and often very indistinct dermal papillae on the dorsal surface of the shanks, and sometimes ankles. These papillae usually disappear completely with the approach of winter.

2. Frogs of the second year, measuring from 26 to 34 mm. in body length.

This period is characterized by normal spermatogenesis and the eventual attainment of sexual maturity. The products of prespermatogenesis disappear during early spring of this year, and the gonial cells then undergo an extensive multiplication, resulting in the production of numerous secondary spermatogonia in cyst formation. These spermatocysts are testicular homologues of oöcysts commonly found in the larval ovary. Cells of the same spermatocysts generally enter maturation at the same time, but in further development, different stages of spermatogenesis are sometimes found in the same cyst. Early spermatocytes are recognized by a number of chromatin knots in their nuclear reticulum.

These knots become gradually changed into distinct spiremes, and the process is accompanied by an increase in nuclear size. At the end of this growth period, the nuclei show many delicate irregular threads with minute thickenings throughout their length. These leptotene threads here, as in oögenesis, soon undergo a process of condensation to form a synizetic mass, which is much more compact than the contraction-figure in oögenesis, and appears to be almost homogeneous with short filaments projecting from one side. In our frog species, synizesis is apparently a definite normal stage in the maturation cycle of the germ cells in either sex. It is not an artifact, as has been claimed by SWINGLE (1921) and others for various other amphibians. In early spermatocyte development, the nucleoli appear as rounded bodies larger than the chromatin net-knots. They soon become obscured, and no trace of them can be found during synizesis or at any of the subsequent stages. In oögenesis, the nucleoli remain identifiable throughout the postsynizetic development.

In May and throughout the summer, the testes have increased in size, and the seminiferous tubules become enlarged. Spermatogenesis is here at the height of its activity. During June and July, the tubules are full of spermatocytes in all stages of synaptic development. Many of the spermatocytes are in synizesis. The thick spiremes (pachynema) evolved from the synizetic mass show no sign of external duality. They undergo a series of changes leading to the complete formation of tetrads arranged on the first maturation spindle. After the maturation divisions, the spermatocytes become transformed into spermatids, which, as soon as formed, undergo spermioteleosis and develop into spermatozoa. During spermioteleosis, the spermatocysts dissolve and the follicular elements of the cyst assume the rôle of SERTOLI cells. In late summer and throughout the fall, the testes show an increasing number of transforming spermatids and spermatozoa. Winter testes contain very few or no spermatocytes, and spermatids, but numerous spermatozoa (fig. 23). The spermatozoa occur in small bundles, with their heads attached to the proto-



Fig. 23. Winter testis of a second-year male frog, 31 $\frac{1}{2}$ mm. in body length. Note abundance of interstitial tissue, and many spermatozoa in the seminiferous tubules.

plasm of the SERTOLI cells, and their tails orientated toward or into the tubule lumen. Scattered along the periphery of the tubule there are a number of primary spermatogonia, readily recognized by their large size, and their oxyphilous polymorphic nuclei. These gonidia are direct descendants of the germ cells of the larval testes, or, more specifically, of those identified as primary spermatogonia in the early post-larval testes. They may be called mother spermatogonia and are kept in reserve to start a new spermatogenetic cycle after the first spawning.

During early October, the interstitial tissue makes its first appearance in the testis. Younger testis of this year or of the previous year, whether normal or undergoing prespermatogenesis, contain in the inter-tubular spaces only a few stroma cells, besides rete elements and blood

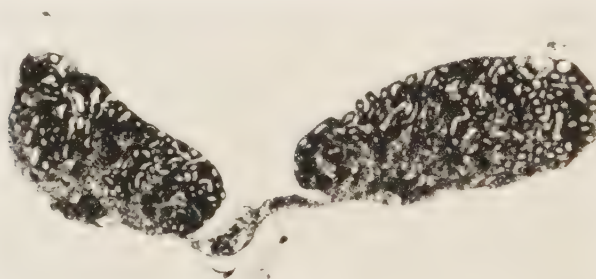


Fig. 24. Postlarval male 22 mm. in body length, killed on May 27th. Section shows rudimentary postgonads and remains of MÜLLERIAN ducts along the lateral sides of the kidney.

vessels. With the approach of winter, the interstitial tissue increases in amount, and during the following breeding season, it becomes dense and compact. Histologically, it appears to be a syncytium of small cells, the nuclei of which are of various shapes with irregular contours, and with deeply staining granular contents. These cells can not be confused with the large, lightly-staining, polymorphically-nucleated primary spermatogonia, nor with the secondary spermatogonia, as the latter cells are always rounded in form, regular in contour and contain distinct nucleoli. Moreover, the secondary gonidia are generally grouped in characteristic cysts.

The rudiments of the oviduct anlagen are often found in male specimens during early seasons of this year. With the onset of spermatogenesis, their degeneration appears to be hastened, and they generally disappear in a short time. Very rarely do they persist as thin strips of modified peritoneum throughout summer and fall (fig. 24). With the approach of sexual maturity, however, all traces of them have vanished.

Young males often show scattered dermal papillae on the dorsal surface of the shins. During the second summer, these papillary outgrowths

are found in abundance around the anal region and on the exposed surface of the shanks and ankles. They disappear early in the fall. Simultaneously with their disappearance, the nuptial pads begin to develop. These have been variously designated as thumb-pads, thumb-cushions, finger-pads, finger callosities, finger tubercles, nuptial excrescences, or merely as glandular swellings on the supplemental thumb of the forelimbs. At first the pads are small and appear non-pigmented. Gradually they assume a grayish color and become enlarged with mucous glands in the dermis and numerous projecting papillae on the surface. They are fully developed during the winter but do not take on a black color until the breeding season.

Correlated, or at least associated, with the enlargement of the nuptial pads, the webs between the toes of the hind-limbs become more fully developed. The web margin originally concave becomes gradually flattened and eventually assumes a convex form. The developmental history of other somatic sex characters require a separate study. It may be mentioned here that the winter frogs of this year possess strong clasping muscles in the arm, which, together with the nuptial pads, serve to aid the male to retain hold of the female during amplexus.

From the above description, it is seen that young frogs at or near the end of the second year have developed mature sperms in their testes as well as secondary somatic characters. Vasa efferentia, vasa deferentia and vesiculae seminales have also attained the completion of their functional development. The frog has thus assumed all features characteristic of the adult male of this species, and they are capable of functioning sexually, the attainment of which capacity marks the termination of the postlarval period.

B. Female Development.

The time duration of the postlarval period in female development apparently varies with different individuals. Sexual maturity may be attained either at the end of the second or third year. Females of the first year measure from 16 to 28 mm. in body length; those of the second year 28—36 mm. Most specimens of the latter age group caught afield during late summer and early fall, show, in their ovaries, numerous large pigmented ova, which will be ready for spawning during the following breeding season. Some females of similar age and captured in the same season, do not, however, show any sign of approaching sexual maturity. These individuals are evidently delayed in development on account of malnutrition or other unfavorable conditions, and apparently do not become mature until the end of the third year.

The ovaries of young postlarval frogs undergo little histological change, and are, in general structure, similar to the fully differentiated larval ovary. Growing ova are found in greater abundance, as oöcysts

become fewer in number. In winter months of the first year, the ovary shows arely synaptic figures, no mature eggs, but numerous ova in the progress of growth. In or near the gonad peritoneum, there are present here and there and almost anywhere, single or small groups of primary oögonia. These gonial cells become active in the following spring, and give rise, by division, to more primary oögonia and new secondary oögonia, which soon begin a new oögenetic cycle. The ova formed during the early larval period are destined for the first spawning. Those formed

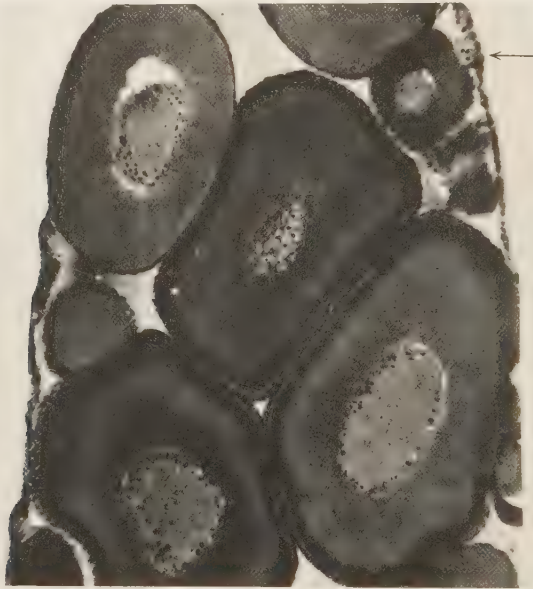


Fig. 25. A second-year female frog, killed on July 16th and measuring 31 mm. in body length. Section of the right ovary to show the follicular and thecal envelopes of the growing ova. Arrow points to a small oöcyst, the germ cells of which are undergoing synizesis.

in late larval development and during the postlarval period furnish the subsequent generations of mature eggs. The process of maturation in postlarval as well as adult ovaries conform fundamentally to the larval type already described. SWEZY (1929), and SWEZY and EVANS (1929) have recently shown that in several mammals, the embryonic ova undergo typical maturation phases, which become greatly modified or even lost in the oögenesis of the germ cells developed during adult life.

As the ova grow in size, the minute cytoplasmic granules found in earlier stages, become indistinct and their future history has not been investigated. The chromatin meshwork becomes more oxyphilic and loosely arranged than previously, until finally it is no longer identifiable as such. Visible chromosomes do not reappear until the ovum is about to be fertilized, at which time maturation divisions take place.

In the large maturing ovum (fig. 25), the peripheral zone (zona radiata) is readily recognizable, forming what may be designated as the vitelline membrane. Enclosing the cells, is a single layer of granulosa or follicle cells, outside of which is the theca folliculi permeated with vascular network. The theca tissue may be one or two cells in thickness, generally only one, in large yolk-laden ova. The theca nuclei are flattened, attenuated, somewhat spindle-shaped. The nuclei of the follicle are at first cuboidal, becoming elongated as the ovum grows in size. In their final form, they appear very similar to the theca nuclei, both of which are arranged with their long axes parallel to the surface of the ovum.

As previously mentioned, the female frog may become mature either in the second or third year. In either case, vitellogenetic activity begins



Fig. 26. Ovaries and oviducts of a newly metamorphosed female frog, 22 mm. in body length, killed on July 16th.



Fig. 27. Female frog killed on January 28th, measuring 21 mm. in body length. Note the foldings of the ovaries and a small ovarian structure behind the right ovary.

during the summer of the respective year. The ova enlarge rapidly, as they become charged with deutoplasmic materials. In the following winter, they have attained the mature size, readily recognizable to the naked eye. Winter ovaries contain, besides mature and immature ova, scattered oögonia, singly or in small masses, which are lineal descendants of the primary oögonia found in younger ovaries, and are in turn the progenitors of new germ cells for subsequent oögeneses after spawning.

The eggs laid by young females in their first breeding season are distinctly smaller than those laid by older individuals. The number of eggs laid at one spawning varies from 200 to 600 or more. The number of mature eggs formed in the ovaries appears to be dependent, in no small measure, upon the environmental, and particularly nutritive condition under which the frog in question was living during the previous year.

The ovaries of newly metamorphosed frogs are large, elongated in size, and much indented and wrinkled in outline (fig. 26). With continued growth of the contained ova, they enlarge, and become distinctly folded and lobed, assuming, thus, the characteristic appearance of the adult ovary (fig. 27). The postgonads persist as rudimentary ligaments of loose tissue in the postlarval as well as adult periods. Mature ovaries are voluminous, packed full of large, heavily pigmented eggs, and occupying most of the body cavity.

In an immature female 21 mm. in body length, there is found in the postgonadal ligament on the ventral surface of the right kidney, a small piece of ovarian tissue (fig. 27), entirely independent of the ovary proper which is normal in every respect. This supernumerary ovary is apparently developed from ectopic germ cells. It is feasible that the presence of accessory ovarian tissues or structures may be responsible for regeneration after extirpation of both ovaries as has been reported in many vertebrates.

In recently metamorphosed females, the oviducts appear as two distinct cords running along the sides of the kidneys and anteriorly to the roots of the lungs. On section, they show a distinct lumen surrounded by a single layer of mesothelial cells, which is embedded in loose connec-

Table 4. *Comparative Review of the Germ Cell Cycle in Rana cantabrigensis.*

Periods in the germ cell cycle	Germ cell terminology	
	Male	Female
1. Period of early segregation	Primordial germ cells	
2. Period of apparent sex indifference	Primitive gonia or indifferent gonia	
3. Period of sex differentiation	Primary spermatogonia	Primary oögonia
4. Period of multiplication		
A. Primary multiplication	Primary spermatogonia	Primary oögonia
B. Secondary multiplication	Secondary spermatogonia	Secondary oögonia
5. Period of maturation (spermatogenesis or oögenesis)		
A. 1st meiotic (heterotypic) division	Primary spermatocytes	Primary oöcytes (Ova)
a) Synapsis phase		
b) Growth phase ¹		
c) Disjunction phase (reduction-division)		
B. 2nd meiotic (homotypic) division	Secondary spermatocytes	Secondary spermatocytes
C. Cell metamorphosis	Spermatids	Oötidis (ovum and polocytes)
6. Period of fertilization	Spermatozoa	Mature ovum

¹ The oöcytes undergo two periods of growth. The first growth takes place during the synaptic phase, following which the primary oöcytes enter the second growth, forming typical ova. In case of male maturation cells, very little growth takes place during the heterotypic prophase. There is no second growth.

Table 5. *Outline of the History of the Germ Cells of Rana cantabrigensis in Relation to the Life Cycle of the Animal.*

Life cycle in years	Periods in life cycle ¹	Length of specimens	Periods in germ cell history	History of germ cell development	
				Male	Female
First Year	Embryonic Larval	3—6 mm 6—50 mm	Period of germ cell origin	Embryonic segregation and migration of primordial germ cells	
			Period of apparent sex indifference	Formation of paired germ ridges; Formation of primordial germ glands; Multiplication of germ cells	
			Period of sex differentiation and development	Formation of testes; Multiplication of spermatogonia	Formation of ovaries; Multiplication of oögonia; Oögenesis: 1. Synaptic period (first growth) 2. Growth period proper (second growth)
Second Year	Postlarval	♂: 16—26 mm ♀: 16—28 mm in body length ♂: 26—34 mm ♀: 28—36 mm in body length	Period of fertilization	Multiplication (prespermatogenesis)	
				Spermatogenesis 1. Synapsis and growth 2. Maturation divisions 3. Spermioteleosis	Yolk formation 3. Maturation ² divisions
				Syngamy of mature gametes; Formation of zygotes.	

¹ Owing to limited space, the various periods in the life cycle cannot be represented with reference to their relative lengths of time.² The female germ cells may mature in the second or third year.

tive tissue. Behind the kidneys, the structures become reduced in size and gradually flattened down into the peritoneum. In January specimens, the oviducts are found to have grown posteriorly to the cloacal region. The ducts are first straight, small and thin-walled. With further growth, they assume a wavy appearance. As soon as yolk is formed in the maturing ova contained in the ovary, they undergo an immense increase in size, and produce characteristic convolutions and well-developed uteri.

The primordia of seminal vesicles are developed in young females as in the male. Figure 28 shows the budding of the primordial vesicles from the lateral wall of the WOLFFian ducts. These vesicles may persist for a considerable period, but they remain rudimentary and eventually degenerate as the frog approaches sexual maturity.

Dermal papillae are found in the majority of the first-year females. They may be scanty, scattered on the exposed surface of the shins and



Fig. 28. Transsection through the WOLFFian and MÜLLERian ducts of a young female frog 21 mm. in body length. Note the anlagen of the seminal vesicles budding from the wall of the WOLFFian ducts.

ankles, or numerous and distributed in the characteristic pattern of the adult females. Frogs of the second year and of subsequent years throughout the adult period, show an abundance of these warts especially during the breeding season. In the typical condition, the dermal papillae are dense around the anal region, and on the upper surface of crura, ankles and the last toes. In their maximal development, they are also found abundantly on the back and the sides of the body up to the level of the tympanic membrane, and only sparsely on the plantar surface, and the upper surface of the thigh.

At no stage of development, has the female developed any structure similar to the nuptial pads of the male. The web margin of the hind-limb is distinctly concave.

C. General Considerations.

1. *Precocious maturation of germ cells.* Prespermatogenesis in juvenile testes of frogs was first described by CHAMPY (1913) in *Rana esculenta*. ALLEN (1918) recorded the occurrence of spermatogenesis and production of sperms in thyroidless tadpoles of *Rana pipiens*, which had retained larval characteristics long after the normal time of metamorphosis.

HOSKINS and HOSKINS (1919) observed similar phenomena in thyroid-ectomized *Rana sylvatica*. WITSCHI (1921) found many spermatocytes but no spermatozoa in the testes of an immature 3-year-old *Rana temporaria*. PARMENTER (1925) reported the presence of spermatozoa in 3 tadpoles and a young frog of *Rana pipiens*, all of which specimens were produced parthenogenetically. SWINGLE (1918, 1921, 1926) found very small immature leopard frogs with sperms in their testes. Recently CHRISTENSEN (1930), also on *Rana pipiens*, observed in metamorphosing males many spermatocytes in early synaptic stages. Frogs killed in the second season showed in their testes all stages of spermatogenesis up to mature sperms.

In *Rana catesbeiana*, SWINGLE (1921—1926) has described an abortive maturation cycle in male larvae, and has frequently found numerous sperms in testes of metamorphosing and recently metamorphosed specimens of both differentiated and undifferentiated strains. He claims that in this species a male maturation cycle occurs annually, beginning with the first year of larval life. He further (1921, 1922) claims it probable that in *Rana pipiens* and other frog species with short larval life, the tadpoles undergo an abbreviated and abortive maturation cycle very early in development. In a later publication (1923), he has expressed as his belief that frog races with direct testis formation might undergo a precocious postlarval sexual cycle.

Prespermatogenesis, hitherto encountered, may be either normal (CHRISTENSEN among others), or abortive (CHAMPY and SWINGLE). In the latter case, the maturation cells invariably degenerate without giving rise to fully-formed spermatozoa. CHAMPY has also observed, during or even before prespermatogenesis, large cells which have assumed the nuclear characteristics of young immature oöcytes, and which he regards as hypertrophied elements in the process of oviform degeneration.

In *Rana cantabrigensis*, prespermatogenesis does not occur during the larval period, but has been sporadically encountered in the postlarval testes. The process of this precocious maturation is not abortive, such as it appears to be in *Rana esculenta* and *Rana catesbeiana*, and is apparently normal as far as morphological evidence shows. All stages of spermatogenesis from early synaptic phases to spermatozoa have been found in frogs, which, in other respects, are sexually immature.

In *Rana sylvatica*, WITSCHI (1929a) states that the first-year males do not show any signs of germ-cell maturation and that the germ cells remain in rapid multiplication as primary(!) spermatogonia. It is shown in our present studies that secondary spermatogonia begin to develop in newly metamorphosed males. The young testes of the postlarval period contain only few primary spermatogonia scattered along the periphery of the testis tubules. Most of the germ cells are secondary

spermatogonia, which normally remain multiplying as such or abnormally undergo precocious maturation as already described and discussed.

In the female, no precocious maturation phenomena have been found during larval and postlarval development. SWINGLE, in a foot-note of his 1921a paper, mentions that he has observed typical tetrad formation and a few first-maturation spindles and chromosomes in oöcytes of female larvae of *Rana catesbeiana*. In another paper published in the same year (SWINGLE, 1921b), it is reported that in bullfrog tadpoles, many of the oöcytes at the beginning of the growth period pass through all the stages of diakinesis and form first maturation chromosomes of very large size. Most of the cells degenerate at the time of spindle formation, and in a few cells the maturation process goes as far as the anaphase of the first division, when the spindle apparatus breaks down. This phenomenon is of great interest and significance with respect to the phylogenetic interpretation of the germ cell history in frogs.

2. *The Development of sex during the postlarval period.* A study of the development of sex in the postlarval period has been much neglected owing apparently to the difficulties involved in obtaining the necessary materials and in aging them for study. Most investigators on the germ cell development of frogs have confined their work either to the larval or to the adult stages. In *Rana cantabrigensis*, we have traced the postlarval history of the germ cells and also of the important sex characters in both male and female. In this species, the primordia of MÜLLERIAN ducts, seminal vesicles and efferent ducts of the gonad are formed early and develop similarly for a certain time in both sexes. In either sex, however, the structures of the opposite sex remain rudimentary and with the approach of sexual maturity, they degenerate and disappear. The appearance of heterosexual characters in normal development of both sexes is a strong embryological evidence for the genetical bipotentiality of sex.

In our wood-frogs, functional maturity is attained at the end of the second year in case of the male, and either second or third year in case of the female. In most other species, the age of frogs at sexual maturity is not definitely known. WITSCHI has claimed that in *Rana temporaria*, sexes do not become mature until the end of the fourth season. In case of *Rana sylvatica*, he (1929a) assumes that the frogs "attain maturity during the third season".

III. Adult Period.

The adult period begins as soon as the frogs become mature and capable of functioning sexually. The length of this period, or rather the duration of the sexually functional life of the adult frogs remains to be further investigated. Adult frogs of both sexes undergo a series of periodical or seasonal changes in their sex constitution, which cycle is repeated annually until the cessation of sexual activity.

A. Male development.

The adult male undergoes an annual spermatogenetic cycle as already outlined for the second-year postlarval testis. During hibernation and the breeding season, the testis contains an abundance of intertubular tissues as well as numerous spermatozoa in bundle formation (fig. 23). After the time of amplexus, the lumina of many rete and seminiferous tubules show disorganized masses of sperms, which have not been shed during mating. The intertubular tissue has begun a regressive change, diminishing rapidly in quantity. The SERTOLI cells are seen as syncytial strands



Fig. 29. Adult frog 41 mm. in body length, killed on May 1st. Transection of the right testis showing numerous synaptic figures and scanty intertubular tissue.

of protoplasm extending from the basement membrane of the testis tubule toward the lumen with their nuclei confined to the tapering tips of the cytoplasmic columns. These cells become eventually detached from the tubule wall and gradually deteriorate with the degenerating sperms. Some of the SERTOLI cells may, however, become transformed into follicular cells which enclose the germ cells or the spermatocysts. The large primary spermatogonia enclosed by delicate cellular follicles, are found either in resting stages or in mitoses. Among them are scattered small cysts of secondary spermatogonia, newly formed by division of the primary gonia. Cells of the spermatogonial cysts either remain in multiplication or soon enter maturation. These processes generally become active after the shedding of sperms during amplexus, but in unmated frogs killed in the latter part of April, they are in full activity

despite the presence of numerous spermatozoa. Old degenerating sperms are occasionally encountered in summer testes, and appear as agglomerations of sperm heads or fragments of such. They can readily be distinguished from the newly formed spermatozoa by their structure as well as by the position they occupy and the relationship they bear to the related tissues of the testis.

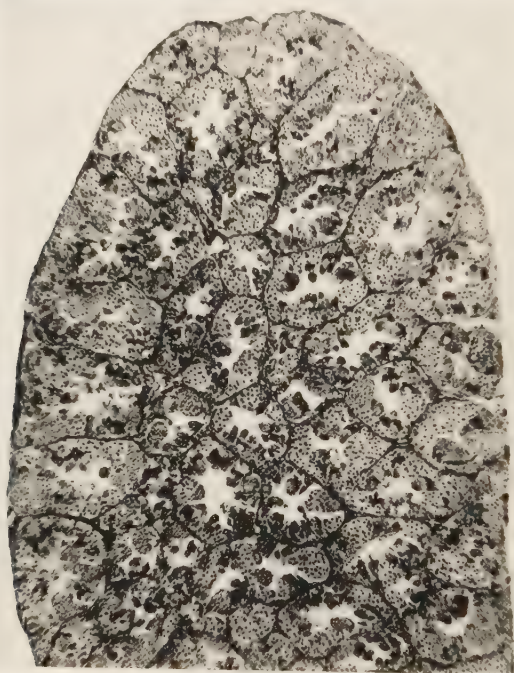


Fig. 30. Adult frog 40 mm. in body length, killed on August 1st. Testis shows all stages of spermatogenesis up to the formation of spermatozoa.

During late spring and early summer, the testis contains many spermatocytes, fewer spermatids and spermatozoa, and only a small number of gonial elements (fig. 29). There are cases, however, in which the maturation cells are relatively few, while the gonial cells show active division to form spermatogonial cysts. This phenomenon appears to indicate that in these testes there might be two waves of spermatogenetic activity, one occurring early in the summer, the other probably in late summer or early fall. Under unfavorable conditions particularly malnutrition, spermatogenesis becomes greatly delayed in time and reduced in the extent of its activity.

Similar to the second-year postlarval testis, the spermatogenetic activity of the adult testis is most intense during the summer (fig. 30),

and is practically arrested in the winter. The intertubular tissue, on the other hand, becomes greatly reduced in quantity after the breeding season, and remains in this condition until late in the fall (figs. 29, 30), when it begins to increase rapidly in amount. During hibernation and the breeding season, the intertubular tissue is at its maximal development. There is, thus, no positive correlation in time between the growth of the intertubular tissue and the maturation activity of the germ cells. The relation may be expressed in a graph as follows:

The solid line represents the activity of spermatogenesis, as estimated by the quantity of spermatocytes and spermatisids; the broken line shows the changes in quantity of the intertubular tissue. The curves are

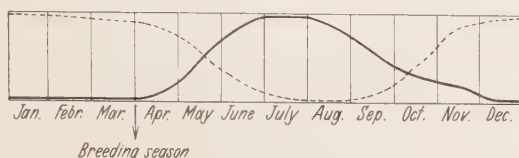


Fig. 31. Graph showing relation between the growth of intertubular tissue and the maturation activity of the germ cells in the male.

constructed on a relative basis, with the highest and lowest points representing the maximal and minimal developments respectively.

Throughout the spermatogenetic cycle, at all times of the year, there are found in the testis tubules a number of mother spermatogonia, which do not participate in the general activity of maturation. These cells form a reserve supply of germ cells for succeeding spermatogeneses. There is no evidence that any of the germ cells are transformed from the surrounding mesodermal cells. All germ cells in the adult testis are derived, either directly or indirectly, from the primary spermatogonia, which, in turn are genetically related to the primordial gonocytes found in early ontogenetic development.

Like the spermatic and interstitial tissues of the testis, the somatic or secondary sex characters of the adult male frog undergo an annual cycle of changes. The male frogs during the breeding season are externally characterized by their enlarged forearms, by their large, pigmented and markedly papillated nuptial pads and by the more or less convex margin of the webs of their hind-limbs. After the breeding period is over, the nuptial pads undergo regressive changes, while the webs of the hind-limbs become less convex than previously, or slightly concave. During this time, the frog develops dermal papillae, which are generally considered as characteristic of the female sex. The papillary outgrowths may be few, and scattered on the dorsal side of the shins, or many, distributed over the posterior part of the back and upper surfaces of the shank, ankle and sometimes the fifth toe. They are best developed during June and July, and disappear early in the fall. Correlatively with their disappearance, the nuptial pads begin to enlarge again, and continue to develop until they assume the functional form. Likewise, the webs of

Table 6. *Reproductive Cycle in Adult Male Frogs.*

Time of Year	Somatic Sex Characters			Testes	
	Nuptial pads	Web-margins of hind-limbs	Dermal papillae	Interstitial tissue	Germinal tissue
Breeding period (late March to early April)	large, pigmented and with marked papillae	flat or slightly con- vex	absent	very abundant	abundance of sperms; mul- tiplication of spermatogonia
Late April	ditto	ditto	ditto	fairly abundant	active multiplication of germ cells; early spermatogenesis
May	small, slightly pigmented and with minute papillae	flat or nearly so	absent or few on the upper surface of shins	scanty	many synzytic and post-synzytic figures
June-July	very small or absent	flat or slightly concave, but less concave than in the female	many on the posterior back and dorsal side of shins and ankles; or few as in May frogs	almost lacking	all stages of spermatogenesis represented; spermatids and spermatozoa in many tubules
August	small as in May frogs	flat or slightly convex	few as in May frogs; or absent	ditto	increasing amounts of metamorphosing spermatids and spermatozoa
September	large, slightly pigmented and with papillae	ditto	absent	ditto	increasing amounts of spermatozoa
October	ditto	ditto	ditto	increasing in amount	ditto
November to March	ditto	ditto	ditto	abundant	spermatogenesis nearly or definitely finished with numerous sperms

the hind-limbs become more fully developed than during the summer, and eventually acquire a flat or a slightly convex margin.

The developmental cycle of the somatic sex characters above described, and its relation to the condition of the testis may be summarized in table 6.

B. Female development.

The ovulation of mature eggs is followed by the formation in the ovary of bodies analogous to corpora lutea, which are composed of disorganized follicle cells in the center surrounded by the theca tissue (fig. 32). These structures soon undergo a more or less gradual regression. The ovary during this period contains a few mature ova which have not been ovulated during spawning, and numerous smaller ova of various sizes. The unextruded eggs and usually some of the immature ova undergo degeneration and become resorbed. The great majority of the small ova remain normal and continue to grow during the following seasons of the year, and most of them become mature for the next spawning. It seems certain, from the postlarval development of the ovary as well as the history of the individual ova, that an oöcyte takes two or three years to mature, generally two under favorable conditions.

During, or immediately preceding, the time of spawning, patches of germ cells appear in the surface peritoneum and occasionally in the septa of the ovary (fig. 32). These germ patches are not developed from peritoneal or other mesodermal elements, but from primary oögonia which are contained in these places. As in the postlarval ovary, the primary oögonia are present in the adult ovary throughout the year. They remain dormant during hibernation, and become active in the

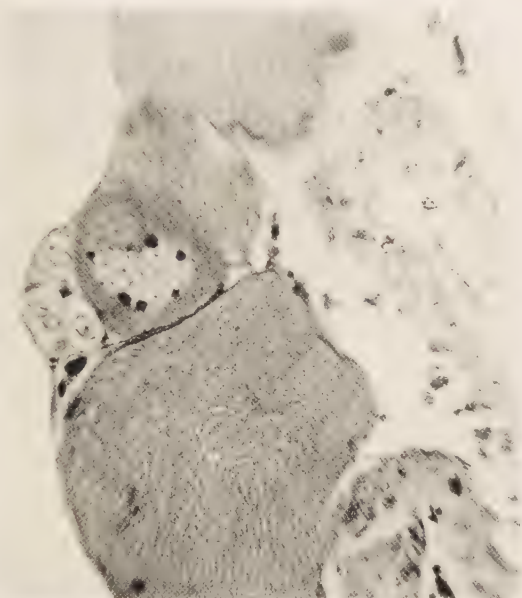


Fig. 32. Section of the adult ovary after spawning. Note a group of oögonial cells (germpatch) in association with small ova, which would become mature for the next spawning. Part of a corpus luteum is seen on the left side of the microphotograph.

spring, giving rise to more primary oögonia and new secondary oögonia. These gonial cells are then grouped into patches. The secondary oögonia later form cysts and soon undergo oögenesis, forming oöcytes. The oöcytes in the adult ovary are similar to those of the larval ovary. In the summer, the new oöcytes are frequently found in the synaptic phases of development (fig. 25). With the approach of cold weather, they have entered the second growth period, forming small ova, which remain inactive over the winter to resume growth the following spring. These ova generally mature in another year.

As regards its external features, the adult ovary during hibernation and before the spawning period is very large, with a considerable number of ripe, pigmented ova in evidence. After ovulation, the ovary shrivels to a small size. During the following months, it gradually enlarges with the progress of oöcyte development, and finally attains enormous dimensions as the contained ova become heavily loaded with yolk materials.

C. General consideration.

1. *Seasonal changes in the testes and in the somatic sex characters of the male.* FRIEDMANN (1898) reported that in *Rana fusca*, *Rana viridis*, *Hyla arborea*, and *Bufo vulgaris*, the development of the interstitial tissue was more or less parallel to the progress of spermatogenesis. The interstitial tissue was most abundant when spermatogenesis was at its height, and less abundant when the latter process was practically arrested. MAZZETTI (1911) confirmed FRIEDMANN's results for *Rana fusca* and *Rana viridis*. CHAMPY (1908, 1913), working on the same species (*Rana esculenta* = *Rana viridis*; *Rana temporaria* = *Rana fusca*), has, however, found that there is no positive correlation between the quantity of the interstitial cells and the intensity of the spermatogenetic activity. In *Rana esculenta*, the interstitial tissue attains its maximal development during the regressive phase of spermatogenesis, and retains this condition until shortly after the breeding season. From July to October, the interstitial cells are very scanty, while spermatogenesis is then most active. In *Rana temporaria*, the interstitial tissue is at its minimum in the fall and winter. In March, which is the breeding period for this species, it gradually increases in amount and is best developed in May and June. From July to October, it undergoes a regressive change, during which time spermatogenesis proceeds rapidly.

In *Rana cantabrigensis*, our data agree in essential points with CHAMPY's findings on *Rana esculenta*. The curve of growth of the intertubular tissue, as seen in figure 31, does not correspond, but is inversely correlated, with the curve of spermatogenetic activity. Table 6 shows that the intertubular tissue decreases in amount after the breeding season, and increases late in the fall when spermatozoa are formed in quantities.

The seasonal modifications of the nuptial pads in the male have been thoroughly investigated in European frogs. The developmental cycle of the nuptial pads in *Rana cantabrigensis*, as summarized in Table 6, is similar to the condition found in other frog species by SMITH and SCHUSTER (1912), and ARON (1926). In our wood-frogs, the developmental condition of the webs of the hind-limbs appears to be correlated with that of the nuptial pads. It is a noticeable fact that the male frog of our species, during the regressive changes of the nuptial pads develop many dermal papillae similar to those found in the female sex. These papillary outgrowths are often found in immature males. Thus, it appears that the fundamentals of the dermal papillae are present in both sexes, but they are in some way inhibited in their development in the male during most of the year. Since the disappearance of dermal papillae is always accompanied by the growth of the nuptial pads, it seems probable that both processes may be induced by the same physiological factor or factor-complex.

Regarding the relationship of the gonad and the secondary sex characters there has been much discussion and dispute. The experimental work of STEINACH (1894, 1910), NUSSBAUM (1909), MEISENHEIMER (1911, 1912), and others, has often been cited as supporting the contention that the development of the secondary sex characters in the male frog is dependent upon the presence of the gonad. This dependence appears to be of a harmonic nature with or without the mediation of the nervous system. SMITH and SCHUSTER (1912), and SMITH (1913), from their work on the removal and transplantation of the gonad in *Rana fusca*, have, however, maintained that there is no sufficient evidence to prove that the testes contain an internal secretion which can influence the growth of papillae of the nuptial pads in a castrated frog.

CHAMPY (1913) is unable to find any correlation between the secondary sex characters and that of the interstitial tissue. In his recent work (1924, 1926), he is inclined to think that the development of the glands of the thumb pads is dependent upon the nutritive condition of the individual and is not a seasonal condition. The hormone responsible for the development of the secondary sex characters is a product of metabolism, and not of any particular tissue in or outside the gonad. ARON (1926), on the contrary, concludes that the interstitial cells constitute the sole source of the hormone governing the secondary sex characters of Anura.

In *Rana cantabrigensis*, there is no correlation between the developmental cycle of the secondary sex characters and that of the spermatogenic tissue of the testes. It is further noted that the increase of the intertubular tissue follows, and does not precede, the development of the nuptial pads. The latter process appears, thus, to be initiated by a certain physiological mechanism, which in actual operation eventually induces

the growth of the intertubular tissue. The intertubular tissue, thus considered, is merely an accessory product of certain formative substances or hormones which regulate the developmental cycle of the secondary sex characters. It is considered probable, however, that the intertubular tissue may function in some way in maintaining the male secondary sex characters, after they have been developed. Disappearance of the intertubular tissue after the breeding season is followed by a gradual regression of the nuptial pads.

2. *Continuity of Germ cells.* Many workers on the development of germ cells in adult amphibians have claimed a somatic origin of these cells. WALDEYER (1870), SPENGEL (1876), VALAORITIS (1879), IWAKAWA (1882), HOFFMANN (1886), SEMON (1891), MCGREGOR (1899), GATENBY (1916), OBRESHKOVE (1924), and HARGITT (1923, 1924) may be cited as examples. Most of these writers worked on females and claim that ova are derived from peritoneal cells, or the so-called germinal epithelium. VALAORITIS believes that the ovum is primitively a white blood corpuscle (leucocyte), which becomes lodged in the epithelium of the ovary and here proceeds to mature. MCGREGOR, OBRESHKOVE, and HARGITT, working on male urodeles have maintained that the large spermatogonia are formed from the epithelial cells which line the collecting ducts or from the peritoneal epithelium which covers the testis. The works of OBRESHKOVE and HARGITT have been critically discussed by POSKA-TEISS (1929).

In *Rana cantabrigensis*, throughout the larval, postlarval as well as adult development, we have emphasized, in different places of our description of materials, the presence of primary spermatogonia and primary oögonia in the male and female respectively. At no stage in the entire life history of either sex, are these gonial cells entirely used up. A sufficient number of them is always kept in reserve to perpetuate the germ line throughout the development of the gonad. These primary gonia, as shown by our embryological evidence, are lineal descendants of the primordial germ cells, and are not derived from any other source. The peritoneal epithelium of the testis or of the testis tubules may contain these gonial cells, but do not proliferate them. The new spermatocysts formed after spawning are not derived from a somatic origin, as HARGITT and others have claimed for the Urodela, but are developed from the actively dividing primary spermatogonia. Likewise, in the ovary the germ patches found during the breeding season are derived from the primary oögonia which are found in abundance in the winter ovary, and not from peritoneal or retro-peritoneal elements, as maintained by GATENBY among others. The constant presence of primary or mother gonia in both sexes and their developmental activities show clearly that they constitute the permanent and exclusive source of germ cells throughout the development of the gonad. The Keimbahn of *Rana*

cantabrigensis is continuous from the embryonic origin of the primordial germ cells.

Summary.

I. Larval period.

A. Male development. 1. In the beginning of testis development, the germ cells, originally located in the cortical region, migrate inward to the rete cords, which have in the meanwhile enlarged and become anastomosed with one another to form a medullary germinal core.

2. Germ cells do not undergo maturation but remain in rapid multiplication as primary spermatogonia.

3. Germ cells and the associated somatic tissues become grouped in testicular ampullae which subsequently develop into seminiferous tubules.

4. The rete tissue becomes organized to form rete efferentia (rete testes), the lumina of which tubules constitute the secondary genital cavity in the testis.

5. No transformation of mesodermal or other somatic cells into germ cells has been observed. All germ cells are lineal descendants of the primordial germ cells.

6. Degeneration and expulsion of germ cells have been sporadically encountered in the testis.

7. The testes remain small, compact and narrow, with a fairly regular contour. The left testis is generally longer than the right one.

8. The progonads develop into fat-bodies, the left one of which always appears earlier, and is better developed than the right one. The postgonads remain small and form elongated rudimentary ligaments.

9. Ectopic germ cells or groups of germ cells have been found in various places near the gonad. Those in the postgonad are generally found in the process of expulsion into the coelom.

10. The extragonadal rete tissue forms the primitive vasa efferentia. Primordia of MÜLLERIAN ducts are developed during metamorphosis. Dermal papillae appear on the exposed surface of the shins of metamorphosing males.

B. Female development. 1. Germ cells generally remain in the peripheral zone or occasionally migrate to the inter-cordal medulla.

2. Germ cells first multiply as primary oögonia, most of which soon become transformed as secondary oögonia. The latter cells are subsequently grouped into oöcysts and begin maturation, forming oöcytes.

3. The peripheral germinal epithelium becomes gradually thickened and forms the definitive ovarian cortex.

4. The rete cords become transformed into ovarian sacs, the lumina of which constitute the secondary genital cavities in the ovary.

5. Throughout larval oögenesis, primary or mother oögonia are always reserved in sufficient numbers to furnish subsequent generations of

maturation cells. There is no evidence for the transformation of somatic cells into germ cells. All germ cells, whether in multiplication or in oögenesis, are genetically related to the primary oögonia, which in turn are derived from the primordial germ cells.

6. Degeneration and exulsion of germ cells have been occasionally observed in the ovary.

7. The ovaries become large and elongated, with an irregular and much corrugated outline. The left ovary is generally longer than the right one.

8. The progonads develop into corpora adiposa, the left one of which, as in the male, always appears earlier and is larger than the right one. The postgonads are short and rudimentary.

9. Ectopic germ cells, singly or in groups, have been found in various places adjacent to the gonad.

10. The extragonadal rete remains rudimentary throughout the entire period. Primitive oviducts and dermal papillae are developed in the metamorphosing females.

II. Postlarval period.

A. Male development. 1. During the first year, the germ cells generally remain in multiplication as primary and secondary spermatogonia. Occasionally they undergo precocious maturation or prespermatogenesis.

2. Normal spermatogenesis takes place during the second year, resulting in the production of mature sperms toward the end of this year.

3. Primary or mother spermatogonia are present in the testes at all stages of testis development. They are reserved as the constant and exclusive source of new germ cells.

4. The testes become oval-shaped, with well-developed fat-bodies in front and small elongated postgonads behind.

5. Oviduct anlagen remain vestigial during the first year and degenerate entirely in the second year. Vasa efferentia become fully developed. Seminal vesicles are formed.

6. Dermal papillae are often found in young specimens. They are found abundantly in the male of the second summer, but disappear in the following fall. During this time, typical nuptial pads are formed and the webs of the hind-limbs become fully developed, assuming a convex margin.

7. The frog becomes mature and sexually functional at the end of the second year.

B. Female development. 1. Oögenesis is in full course, except during hibernation. Vitellogenetic activity begins in the second or third summer, and mature ova are formed during the following winter.

2. Primary or mother oögonia are always present and constantly give rise to more primary oögonia or to new secondary oögonia which initiate new oögenetic cycles.

3. The ovaries become voluminous after yolk formation in the contained ova. Fat-bodies are large in size, while the postgonads remain short and rudimentary.

4. Primordial seminal vesicles are developed but they later degenerate. MÜLLERIAN ducts become differentiated as functional oviducts.

5. Dermal papillae are found in some first-year females, and are well developed in females of the second year and of subsequent years throughout the adult period.

6. The frog becomes sexually mature either at the end of the second or third year.

III. Adult period.

A. Male development. 1. The germ cells undergo an annual spermatogenic cycle. The intertubular tissue likewise undergoes an annual cycle of changes.

2. The changes in the quantity of the intertubular tissue is inversely correlated with the intensity of spermatogenic activity.

3. Primary spermatogonia are present at all times of the year. They are direct descendants of the primordial germ cells and are in turn the progenitors of germ cells for annual spermatogenesis. The germ line is continuous.

4. The secondary sex characters also undergo an annual developmental cycle. Dermal papillae are developed during the summer and disappear as the nuptial pads begin to develop in the fall.

5. The developmental cycle of the secondary sex characters does not appear to depend on the spermatogenic or intertubular tissues of the testis.

B. Female development. During the breeding season, mature eggs are ovulated. Small, immature ova remain in the ovary and most of them become mature for the next spawning. It takes two or three years for an oöcyte to mature, depending apparently on the nutritive condition.

2. Primary oögonia are present in the adult ovary at all times of the year. They constitute the sole and exclusive source of the female germ cells. The germ line in this sex, as in the male, is continuous.

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